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CONTENTS

PAGES

1. Mitochondria and Golgi Bodies in *Endamoeba gingivalis* (Gros) Brumpt, by David Causey; plates 1–3 .... 1– 18
2. Mitochondria in *Leishmania brasiliensis* Vianna, 1911, by David Causey; plate 4 .... 19– 28
3. Mitosis in *Ceratium hirundinella* O.F.M., with Notes on Nuclear Phenomena in Encysted Forms and the Question of Sexual Reproduction, by Richard P. Hall; plates 5–9, 5 figures in text 29– 64
4. The Cultivation of *Endamoeba gingivalis* (Gros), by Beatrice Fay Howitt; plates 10–12, 4 figures in text, 3 charts 65–126
5. The Behavior of *Endamoeba dysenteriae* in Mixed Cultures with Bacteria, by Charles A. Kofoid and Edna Hannibal Wagener; plates 13–15 127–154
6. Studies of the Effects of Certain Drugs upon *Endamoeba dysenteriae in vitro*, by Charles A. Kofoid and Edna Hannibal Wagener 155–166
7. Some New and Some Previously Unreported Hydroids, mainly from the California Coast, by C. McLean Fraser; 7 figures in text 167–172
8. The Effect of Certain Drugs and Dyes upon the Growth of *Endamoeba gingivalis* (Gros) *in vitro*, by Beatrice Fay Howitt 173–182
9. Experiments with *Endamoeba gingivalis* (Gros) in Mixed Bacterial Cultures; in Filtered Saliva; on a Solid Base; with Peritoneal Cells; and with Digestive Secretions, by Beatrice Fay Howitt; plates 16–17 183–202
10. On *Oxyphysis oxytoxoides* gen. nov., sp. nov., a Dinophysoid Dinoflagellate Convergent toward the Peridinioid Type, by Charles A. Kofoid; plate 18 203–216
11. Mitochondria in *Euglena gracilis* Klebs, by David Causey; plates 19–20 217–224
12. Mitochondria in *Noctiluca scintillans* (Macartney, 1810), by David Causey; plate 21 225–230
13. Mitochondria in Ciliates with Especial Reference to *Paramecium caudatum* Ehr., by David Causey; plates 22–24 231–250
14. Studies on the Ingestion of Leucocytes, and on Mitosis, in *Endamoeba gingivalis*, by Horace J. Child; plates 25–29, 9 figures in text 251–284
15. On *Oxymonas*, a Flagellate with an Extensile and Retractile Proboscis from *Kalotermes* from British Guiana, by Charles A. Kofoid and Olive Swezy; plate 30 285–300
16. On *Proboscidiella multinucleata* gen. nov., sp. nov., from *Planocryptotermes nocens* from the Philippine Islands, a Multinucleate Flagellate with a Remarkable Organ of Attachment, by Charles A. Kofoid and Olive Swezy; plates 31, 32 301–316
17. On the Cestode Genus *Dipylidium* from Cats and Dogs, by Theresa Marie Millzner; plates 33–39 317–356

PAGES

18. A Useful Modification of a Clearing Fluid Formulated by Spalteholz, by Franklin P. Reagan 357–359
19. The Earliest Blood Vessels of the Mammalian Embryo, Studied by Means of the Injection Method, by Franklin P. Reagan 361–364
20. The Biological Relationships of ***Leishmania*** and Certain Herpetomonads, by Edna Hannibal Wagener and Dorothy Ann Koch; plates 40–43 365–388
21. Notes on Termites from Arizona, with Descriptions of Two New Species, by Thomas E. Snyder; 6 figures in text 389–397
22. Copelata from the San Diego Region, by Christine E. Essenberg; 170 figures in text 399–521
23. Observations on Gradual Disintegration and Death of Copelata, by Christine E. Essenberg 523–525

Index 527–532

MITOCHONDRIA AND GOLGI BODIES IN ENDAMOEBA GINGIVALIS (GROS) BRUMPT

BY

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CONTENTS

PAGE
Introduction.......... 1
Technique.......... 3
Morphology of the motile phase.......... 4
Cytoplasmic inclusions.......... 4
  Mitochondria.......... 4
  Golgi apparatus.......... 5
  Fat bodies.......... 6
Functional significance of mitochondria.......... 7
  Amoeboid movement.......... 8
Spiral organization.......... 10
Summary.......... 11
Literature cited.......... 12
Explanation of plates.......... 14

INTRODUCTION

The varied activities which are constantly present in the cell comprising the organization of a protozoan make the Protozoa peculiarly well adapted for the study of the cytoplasmic inclusions. The various vital functions are all to be found, and, what is of greatest importance, in varying degrees of completion, in the single organism. Thus, in a large and active amoeba one finds food vacuoles in all stages of their metabolism from the newly ingested material to the final stages immediately preceding the complete disappearance of the vacuole itself. For a study of certain fundamental processes, therefore, such as the relation of the cytoplasmic inclusions to metabolism, a protozoan cell offers more possibility of success than a metazoan cell.

The lack of means for direct experimentation and critical analysis of cytoplasmic inclusions, particularly of the mitochondria, has resulted in a considerable literature dealing with their morphology and distribution, and a number of theories as to their function. Several of these hypotheses have been concerned with the function of the mitochondria: (1) that they are the basic living elements (Altmann, 1890); (2) that they are symbiotic organisms which are either bacteria or very similar to bacteria (Wallin, 1922); (3) that they are concerned with histogenesis (Meves, 1918); (4) that they are similar to plant plasts and elaborate complex materials from the surrounding cytoplasm (Regaud, 1909); and (5) that they have to do with the respiration of the cell (Kingsbury, 1912).

To these various theories numerous investigators have added modifications and alternatives. Altmann's idea of vital units suspended in a non-living matrix no longer receives support, and it has been replaced so far as its theoretical side is concerned by the modern conception of the 'organism as a whole.' The idea that mitochondria are bacteria, or similar organisms, advocated by both Portier and Wallin, has not been favorably received in spite of Wallin's considerable evidence to show that they are bacteria. If mitochondria were proved to be bacteria, Wallin's view that they are symbiotic forms would require proof of its correctness; the mere association of organisms, however close, does not constitute symbiosis.

The presence of mitochondria in an organism so devoid of structural differentiation as an amoeba makes the possibility of their being concerned in histogenesis rather unlikely. Similarly, in organisms as minute as the Protozoa, a special mechanism for cellular respiration is hardly necessary. Their uniform distribution in practically all living cells, in all that have been investigated, makes it seem very probable that they are associated with the most fundamental process going on in living cells—that of metabolism.

A great amount of cytological work in recent years has been on the mitochondria in metazoan tissues, largely descriptive, but with some experimental work along the lines of pathology. Wallin's culture experiments are an outstanding exception to the general type of work. Fauré-Fremiet (1910) has made the most extensive investigations on the mitochondria in the Protozoa, a comprehensive study of the group as a whole. While his technique for prepared material is adequate from a more modern standard, his use of vital stains such as neutral red, methylene blue, and Bismarck brown is questionable, as none of these is as specific as the Janus green B. More recently Alexeieff (1917) has studied a number of flagellates with reference to their mitochondria. A few shorter papers have appeared—Arndt (1914), Hirschler (1914), Vonwiller (1915), Shipley (1916), but the total amount of work on the Protozoa with reference to their cytological inclusions has been surprisingly small.

The present paper deals in particular with the distribution and function of the mitochondria and their relation to the other cytoplasmic inclusions in the amoeba of the mouth, *Endamoeba gingivalis.* It was undertaken at the suggestion of Dr. C. A. Kofoid, under whose direction it has been carried on, and to whom I am indebted for the many suggestions and criticisms which made it possible. Dr. Olive Swezy has aided throughout the work in matters of technique and interpretation.

TECHNIQUE

The amoebas have been studied both in the living condition when vitally stained with Janus green B, Sudan III, and Scharlack R (Harxheimer), and in fixed material. They have in all cases been obtained from the subgingival spaces of my own mouth. A dilution of 1/100,000 of Janus green B was used for the study of the living amoebas. The exudate was added to Janus green B and studied immediately. For permanent preparations the exudate was mixed with saliva on a slide and fixed with osmic acid vapor for one hour, and then stained, either with Regaud's iron-haematoxylin method for blood cells (Cowdry, 1918, p. 61), or by the Bensley-Cowdry acid fuchsin and methyl green method. For the haematoxylin method the smears, after being washed in distilled water, were put in 3 per cent potassium dichromate for a number of hours, washed in distilled water, and mordanted for from 30 to 40 minutes in 5 per cent iron alum. They were then stained in the modified haematoxylin for 30 to 45 minutes. The potassium dichromate solution may be omitted. The Bensley-Cowdry method was used as follows: The material fixed in the osmic acid vapor was washed in distilled water and mordanted for from 24 to 36 hours in 3 per cent potassium dichromate. It was then washed in distilled water and flooded with acid fuchsin. This was heated until steaming, cooled for six minutes, and washed with distilled water. It was then flooded with methyl green for from 1 to 2 minutes, plunged into 90 per cent alcohol for a second or two, dehydrated in absolute alcohol, and cleared in xylol. With such fixation and staining nuclear details are almost wholly lacking, but the cytoplasmic details are very good; the cytoplasm, however, lacks the clear transparency given by the hot Schaudinn method. The material was checked by similar material fixed in the hot Schaudinn fixing fluid.

MORPHOLOGY OF THE MOTILE PHASE

The cytology of *E. gingivalis,* as seen in the living condition when studied in physiological salt solution and iodine eosin solution, and when prepared by fixing in Schaudinn's fluid and staining with iron haematoxylin, has been adequately described by Kofoid and Swezy (1924).

The amoeba is found almost exclusively in the gums, and may readily be pressed out from the subgingival groove, being found in the exudate composed largely of broken-down cells and bacteria. In physiological saline solution or saliva, it exhibits clear, hyaline ectoplasm and granular endoplasm, has clear pseudopodia, and a definite pellicle. The nucleus shows, after fixation with Schaudinn's fluid, a definite morphology upon which specific identity is based (Kofoid and Swezy, 1924). The food utilized is of two kinds: principally leucocytes (salivary corpuscles), of which, apparently, only the nuclei are utilized; and, less commonly, the various types of bacteria found in the mouth. The food bodies are always found in vacuoles which vary in size from scarcely larger than the contained food particles to sizes much greater than that of the food particles. This difference in the size of the vacuoles is more pronounced in fixed material, material fixed with Schaudinn's fluid commonly showing larger vacuoles than that fixed with osmic acid vapor.

CYTOPLASMIC INCLUSIONS

The cytoplasmic inclusions present in the amoeba are the food vacuoles, the Golgi elements, the mitochondria, and the fat bodies, all of which, with the possible exception of the Golgi elements, are intimately associated with the metabolic activities of the organism.

MITOCHONDRIA

The mitochondria present are typical in size and morphology, resembling those of metazoan tissues. They vary somewhat more in length and diameter than those of metazoan tissues, and show more intermediate sizes. The number present in an amoeba varies with the metabolic activity of the amoeba, and to some extent the morphology varies with the activity, as will be shown for amoeboid movement. In an active amoeba nearly free from food vacuoles the mitochondria are uniformly distributed throughout the cytoplasm (pl. 1, figs. 1, 8). In an amoeba containing numerous food vacuoles the mitochondria are found more or less intimately associated with the food vacuoles (pl. 1, figs. 2, 9; pl. 2, figs. 11, 14, 16, 17). They are less numerous about the vacuoles in the early stages of assimilation, and more numerous in the later stages; and may, in the final stages, when the vacuole is nearly or quite gone, preserve the shape of the vacuole in their arrangement.

GOLGI APPARATUS

Golgi elements have been reported in the Protozoa by Hirschler (1914) for *Monocystis ascidiae,* and by King and Gatenby (1923) in an *Adelea.* In both forms the Golgi elements appear as crescents, irregular or regular circular bodies, and in the *Adelea* in the irregularly coiled mass characteristic of the metazoan Golgi apparatus, the various shapes being developmental stages. The following confirms and extends the previous work.

As is shown later in this paper, the fate of the food vacuoles varies. Usually the contents are absorbed, and the wall of the vacuole gradually fades away, its position then being indicated roughly by the mitochondria in that area. More rarely there appears on a side of the wall of the vacuole a concentration of material, while the other side disappears. This process results in crescent-shaped bodies lying free in the cytoplasm (pl. 1, figs. 6, 9; pl. 2, figs. 12, 15; pl. 3, figs. 36, 37). These stain intensely, and may show irregular thickenings on the rim (fig. 12). It seems probable that from these irregular thickenings the occasional irregular bodies found in the cytoplasm are derived. But the thickened rim, itself, which appears on the wall of the vacuole, has another fate. By elongation, irregular, more or less closed structures are produced and, in some cases, typical examples of the Golgi apparatus of metazoan cells are to be found (pl. 1, fig. 7; pl. 3, figs. 38–42). These are found after osmic acid fixation, and never after fixation with the Schaudinn's fluid. Problematical structures may appear (pl. 1, fig. 3) in clearly abnormal individuals.

The possibility of interpreting masses of bacteria as Golgi apparatus is unlikely, although the smears have numerous bacteria in them, and these, particularly the spirilla, are occasionally found in masses which superficially resemble Golgi bodies; such masses are always outside the amoeba. In all cases, the individual organisms composing such masses may be resolved by focusing. Bacteria, when within the amoeba, are in my own experience, always in vacuoles (pl. 1, figs. 4, 6). The structures interpreted above as Golgi bodies are never in vacuoles; they are continuous, i.e., cannot be resolved into separate elements; and are not found in material fixed in Schaudinn's fluid, which preserves bacteria equally as well as the technique used for this study. There is a perceptible difference in stainability between these structures and the bacteria present, the former are black, and the latter blue.

The origin of the Golgi apparatus from a region of intense metabolic activity, the food vacuole wall, favors the view that the Golgi elements, regardless of their form, take part in the cellular metabolism in the amoeba. This region, in which the essential digestive enzymes are elaborated, may persist to further the ultimate utilization of the products of vacuolar digestion, particularly if the reversibility of enzyme effects is applicable to protozoan physiology. The known association of the Golgi apparatus with the nucleus favors this view.

Bensley (1910), after tracing the development of the plant-cell vacuole from a system of clear canals closely resembling structures found in the metazoan cell, suggested "only as a working hypothesis, that the network of canals found in so many animal cells is the physiologic and morphologic equivalent of the vacuolar system of the plant cell." This conception has also been suggested by Guilliermond and Mangenot (1922) in their studies of the Golgi apparatus in barley cells. That the clear canals of animal cells correspond to the Golgi apparatus demonstrated in fixed material has been shown by Cowdry (1924) to be true for the cells investigated. There is a growing probability that the Golgi bodies found in living cells are homologous structures, but additional evidence is necessary to demonstrate it as a fact.

FAT BODIES

The fat bodies are found scattered throughout the cytoplasm, usually more or less spherical in shape, and of a size intermediate between food vacuoles and mitochondria (pl. 1, fig. 4; pl. 2, figs. 16, 17). They are in vacuoles which are often of so near the same size as that of the fat body as to be nearly or quite invisible. These fat bodies are dissolved away after fixation with Schaudinn's fluid, and the resulting clear spaces are the cause of the greatly vacuolated appearance of the cytoplasm, as was suggested by Kofoid and Swezy (1924). They stain intensely with both Sudan III and Scharlack R (Harxheimer), and in the material fixed in osmic acid vapor are rendered insoluble. Depending upon their chemical nature they either blacken with the osmic acid or stain in the following stages of the technique.

FUNCTIONAL SIGNIFICANCE OF MITOCHONDRIA

The functional significance of the mitochondria in the amoeba is at least twofold, dealing first, with the metabolic activities centering about the food vacuole, and secondly, with amoeboid movement.

The newly ingested leucocyte nucleus is a spherical or spheroidal body, always in a vacuole, and staining intensely with either haematoxylin or acid fuchsin, in either case being quite black (pl. 3, figs. 19, 20). As it undergoes digestion it loses this stainability more and more, and successively stains dark red, lighter red, and pink with acid fuchsin, or comparable shades of blue with haematoxylin. In the last stages, when the contents are fully absorbed, the vacuole shows as a lighter region than the surrounding cytoplasm. No mitochondria are found about the newly ingested food body (pl. 3, figs. 19–22). This condition continues until the food body begins to lose its stainability in limited parts, which probably represents liquefaction in that portion of the food body. On the wall of the food vacuole in these regions of early liquefaction the first mitochondria appear (pl. 3, figs. 23–25). The process continues until the whole food body stains uniformly lighter in color and is completely liquefied (pl. 3, figs. 26–29), during which period the number of mitochondria increases. In the final stages of absorption of the contents of the vacuole the close association of the mitochondria and the wall of the food vacuole is maintained (pl. 3, figs. 30–33) and when the vacuole as such can no longer be demonstrated the mitochondria are still found in positions indicating the size and shape of the vacuole (pl. 3, fig. 34). The mitochondria are closely applied to the wall of the vacuole, and may conform to the curvature of the wall itself. The wall of the vacuole may fade out uniformly; this appears to be the usual fate of the vacuole. In numerous cases, however, the wall of the vacuole gradually fades away on one side, and material is concentrated on the remaining portion, so that crescentic structures are left in the cytoplasm. Their significance has been discussed above. While little can be said as to the actual time involved in the process of assimilation of the contents of the vacuole, the preponderance of the darkly staining vacuoles indicates that the time for complete liquefaction is relatively long, while that for absorption and the disappearance of the vacuole is much shorter.

The fat bodies appear to result from the clumping together and later fusion of the mitochondria. Careful study shows various stages, often present in a single amoeba, from the close association of the mitochondria, through the various clumpings and fusion, to the spherical fat bodies (pl. 3, fig. 35). Many of the fat bodies which appear spherical can be shown by careful focusing to be irregular and to have the appearance of clumped mitochondria. The fat body appears to be absorbed by the surrounding cytoplasm without any diminution in size. The stainability is gradually lost, the body becoming punctate, and finally free from stainable material (pl. 3, fig. 35). Clear, spherical bodies representing the final stage in the absorption are found in the cytoplasm (pl. 2, figs. 12, 13, 18).

AMOEBOID MOVEMENT

The rôle of the mitochondria in amoeboid movement is readily demonstrated by vital staining with Janus green B. The amoeba under such conditions appears quite different from its appearance in physiological saline solution. According to Kofoid and Swezy (1924),

> when in locomotion *Endamoeba gingivalis* normally exhibits both in life and in stained preparations a division of its substance into a clear, peripheral ectoplasmic zone and a granular, more deeply staining endoplasm containing food vacuoles and nucleus. In life the ectoplasm is crystal clear and hyaline.

This is readily confirmed by establishing similar conditions. When, however, the amoeba is stained with Janus green B, quite a different appearance is found. Almost instantly the cytoplasm is found to be filled with spherical bodies which stain bluish-green, characteristic of mitochondria in other tissues, e.g., leucocytes on the same slide. In fresh preparations the amoebas are rounded up and motionless. If such an amoeba is observed it is found that sooner or later, a matter of a few minutes at most, Brownian movement appears in the mitochondria of some restricted portion of the ectoplasm. At first, this movement may be limited to three or four mitochondria, but soon it spreads to include a number of them. At this time, a pseudopodium begins to form, always in the region of the Brownian movement. By watching for the Brownian movement one can determine the point at which a pseudopodium will form before there is any other evidence of it. As the pseudopodium develops the Brownian movement becomes very pronounced and the mitochondria are seen to advance with the developing pseudopodium, maintaining the rapid movement. As soon as the pseudopodium has reached the greatest extent in its formation, the Brownian movement shown by the mitochondria ceases instantly, 'freezing' in the position reached at that time. Up to this time the mitochondria have been only in the ectoplasm of the pseudopodium, but with the change from a sol to a gel the ectoplasm becomes endoplasm, in which they remain. While the pseudopodium is forming and is in the sol condition, the remainder of the amoeba is in the gel condition, and no Brownian movement of the mitochondria is to be seen. A similar process occurs when a pseudopodium is withdrawn. Amoebas under such conditions remain alive and active for a considerable period of time, up to a half-hour on a cold stage, and show four, five, and even six pseudopodia forming at a time. The change from a gel to a sol and back again is very clearly shown in the behavior of the mitochondria, and takes place in cytoplasm which in saliva or physiological saline solution is remarkably clear and without granules.

The behavior of the mitochondria in this case offers additional evidence of their association with metabolic activity. The phenomenon of amoeboid movement appears to be one of solation taking place about the mitochondria, with a reversion to a gel condition upon the cessation of the movement. If the remainder of the amoeba follows the pseudopodium the mitochondria in the moving portions successively show the Brownian movement, and if the pseudopodium is withdrawn the Brownian movement of the mitochondria continues throughout the process. Amoeboid movement in pus cells on the same slide shows the same Brownian movement of the mitochondria, although the chilling effect of the unwarmed slide prevents any appreciable movement. Usually in such cells the Brownian movement becomes very active, then ceases for a time, and then starts anew without any noticeable movement.

The spherical mitochondria are not so numerous in the fixed material as in the active material, but in forms showing pseudopodia they are to be found (pl. 1, fig. 4). In the fixed material the rod-shaped mitochondria are the prevalent type. The action of the osmic acid vapor is slow enough to permit the amoebas to round up, so that pseudopodia are not commonly found in fixed material. The rod-shaped mitochondria are always associated with the food vacuoles or in the intervening cytoplasm, and the spherical mitochondria with the pseudopodia. The change from the rod-shaped to the spherical shape probably is the result of rapid fragmentation, and for the purpose of increasing the surfaces of the individual mitochondria.

Fauré-Fremiet's observations (1910) on what he doubtfully considered to be *Amoeba gorgonia* Penard are somewhat different. He found the mitochondria in the endoplasm only, and the pseudopodia were clear when formed. After the formation of the pseudopodium a wave of endoplasm containing the mitochondria followed. His use of various vital stains such as neutral red, cresyl violet, Nile blue, and methylene blue renders his observations on the living amoebas somewhat uncertain, as none of them is so specific for mitochondria as the Janus green B.

The functional significance of the mitochondria in the amoeba appears to be one of active metabolism, in which they play the part of plasts, as was suggested by Regaud (1909). They first appear in the vicinity of the food vacuoles, applied to the wall of the vacuole, and increase in numbers as the contents liquefy and lose their stainability. They are active also in amoeboid movement. No indication of division of the mitochondria has been seen, nor any evidence that they have an identity which persists throughout the life of the cell. Work now in progress indicates that the association of mitochondria with food vacuoles is true also in the case of *Paramecium caudatum.*

SPIRAL ORGANIZATION

Schaeffer (1920) has found evidence of a spiral organization in the amoeba, as shown by their locomotion. Kofoid and Swezy (1924) have shown that for *E. gingivalis* "the formation of pseudopodia may continue in rapid succession with no locomotion at all," and while there was no evidence of permanent polarization,

> prolonged locomotion in a single direction can often be seen, and repeated successive formation at or near one pole, with an approximation of equality in the number of pseudopodia formed at the right and left which might suggest polarity and a spiral organization and behavior.

With amoebas vitally stained with Janus green B this is readily confirmed, the mitchondria because of their striking visibility aiding in following the changes in form. Not only are the alternate right and left pseudopodia formed as they have been reported, but intermediate pseudopodia are found, and it is evident that they are formed along a short spiral. The location of the mitochondria in a large amoeba filled with numerous food bodies and fat inclusions gives little morphological evidence of spiral organization. This is due probably to distortion caused by the inclusions. In a small amoeba, however, which is empty or nearly so of food vacuoles, the arangements of the mitochondria is suggestive of such spiral arrangement (pl. 1, fig. 1). The spiral as shown by the mitochondria appears at the border which is thinnest, standing out clearly in the nearly invisible cytoplasm. From this point, probably that of the latest pseudopodial formation, the mitochondria follow the contour of the amoeba, the spiral ending in the vicinity of the nucleus. That this is not a chance distribution is made unlikely by the almost constant distance between the individual mitochondria along the spiral.

SUMMARY

1. Mitochondria, Golgi elements, and fat bodies are demonstrated in *Endamoeba gingivalis*.

2. The mitochondria take part in the cellular metabolism and are intimately associated with both the food vacuoles and amoeboid movement. They appear around the food vacuoles during the digestion of the contents of the vacuoles, and remain after the disappearance of the vacuoles. Such mitochondria are rod shaped. In amoeboid movement the mitochondria appear to initiate the formation of pseudopodia by solation of the cytoplasm in the region of pseudopodial formation, and accompany them during protrusion and retraction. Under such conditions the mitochondria are spherical.

3. The mitochondria are probably metabolic products, playing the part of plasts as suggested by Regaud. No evidence is found for considering the mitochondria to have a persistent identity during the life of the organism.

4. The Golgi elements are probably derivatives of the region surrounding the food vacuoles and metabolic in function.

5. The probability of spiral organization in the amoeba is indicated by the arrangement of the mitochondria under certain conditions.

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Transmitted May 1, 1925.

EXPLANATION OF PLATES

Figures of *Endamoeba gingivalis* (Gros, 1849), Brumpt, 1913, from smears fixed in osmic acid vapor, and stained on plate 1 with Regaud's modified iron haematoxylin, and on plate 2 with Bensley-Cowdry's acid fuchsin methyl green. × 2500 except on plate 3.

PLATE 1

Fig. 1. Amoeba nearly free from food bodies, with mitochondria in spiral arrangement.

Fig. 2. Amoeba with large mitochondria and last stages of two vacuoles.

Fig. 3. Small moribund amoeba with problematical cytoplasmic inclusions.

Fig. 4. Active amoeba with pseudopodium. Note spherical mitochondria in pseudopodium.

Fig. 5. Amoeba with few mitochondria, containing bodies which resemble chromatoidal bodies, but with no definite cyst wall.

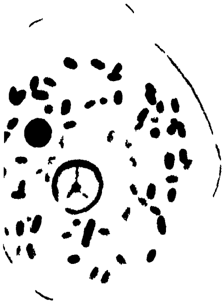
Fig. 6. Amoeba with nearly empty cytoplasm containing sparsely distributed mitochondria, a Golgi element, and one large bacillus in vacuole.

Fig. 7. Small, active amoeba with numerous inclusions and well defined Golgi apparatus near the nucleus.

Fig. 8. Small amoeba with cytoplasm empty of food vacuoles, with mitochondria roughly approximating a spiral.

Fig. 9. Large amoeba containing numerous food vacuoles, mitochondria and a Golgi element.

Fig. 10. Small amoeba with numerous inclusions and irregular problematical bodies in center.



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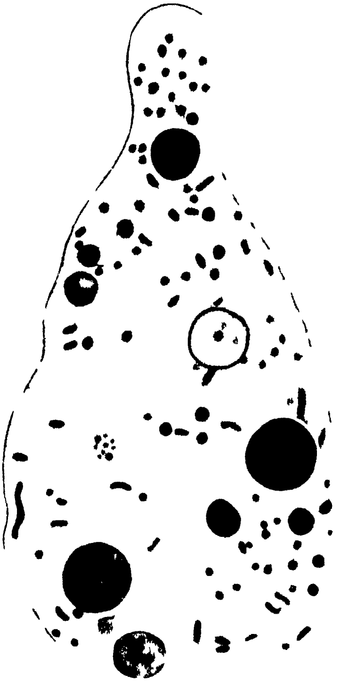

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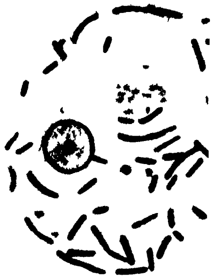

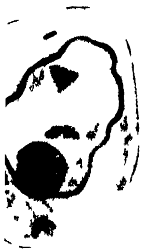

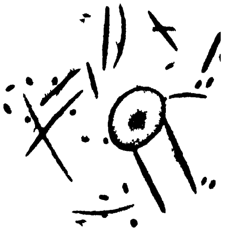

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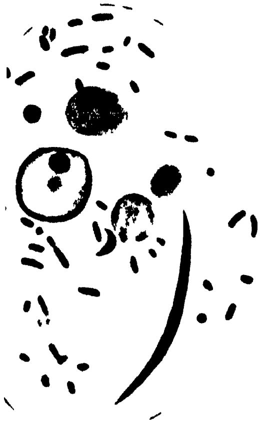

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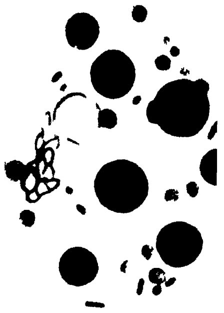

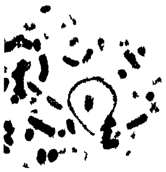

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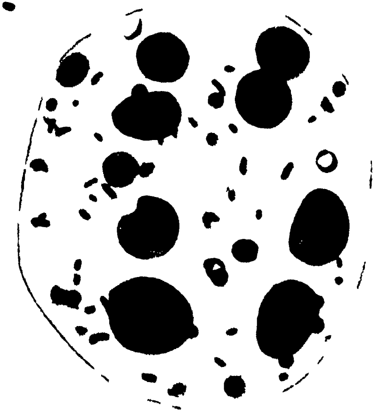

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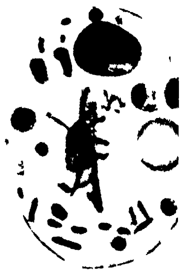


PLATE 2

Fig. 11. Amoeba with numerous inclusions and filamentous mitochondria.

Fig. 12. Amoeba with numerous inclusions. Note formation of crescents on vacuole walls.

Fig. 13. Small amoeba with few inclusions and few mitochondria.

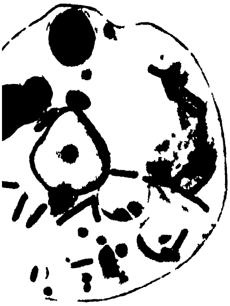
Fig. 14. Amoeba with vacuoles in final stages. Note numerous mitochondria throughout cytoplasm.

Fig. 15. Small amoeba showing formation of crescents on vacuole walls.

Fig. 16. Amoeba with numerous vacuoles in various stages and fat bodies.

Fig. 17. Large amoeba with many inclusions. Note association of mitochondria with food vacuoles.

Fig. 18. Amoeba showing extrusion of food material from vacuole. Note numerous empty vacuoles from which fat has been absorbed.



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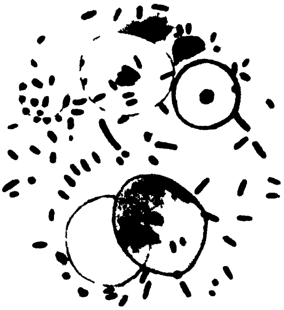

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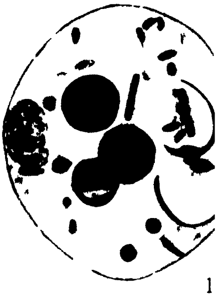

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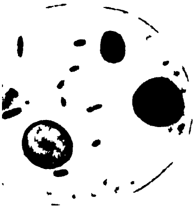

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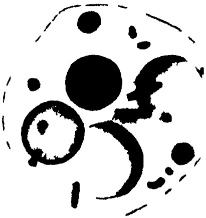

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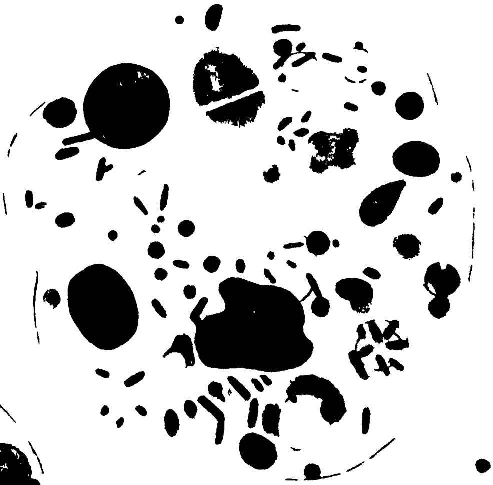

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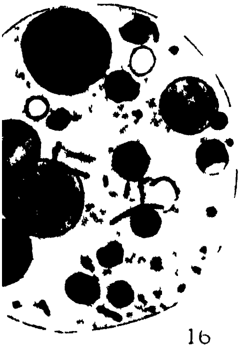

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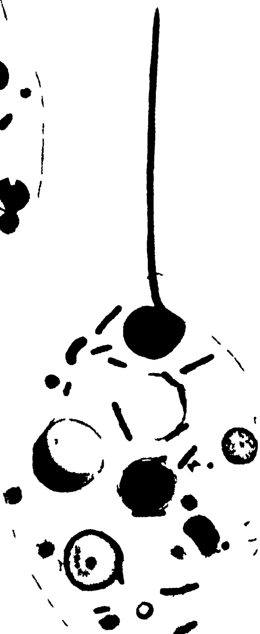

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PLATE 3

Selected vacuoles to show changes during metabolism, and cytoplasmic inclusions showing formation of fat bodies and Golgi elements. × 3400.

Figs. 19–21. Newly ingested nuclei of leucocytes. Note dark color and absence of mitochondria.

Figs. 22–25. Vacuoles showing liquefaction. Note appearance of mitochondria in regions of lighter staining.

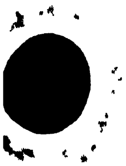
Figs. 26–29. Vacuoles completely liquefied. Note general increase in number of mitochondria.

Figs. 30–33. Final stages in absorption of vacuole contents. Note uniform lighter color.

Fig. 34. Mitochondria showing position of vacuole after the vacuole itself has disappeared.

Fig. 35. Stages showing formation of fat body from mitochondria by clumping of the latter, and the ultimate absorption.

Figs. 36–42. Stages showing formation of Golgi apparatus from crescents lying free in cytoplasm.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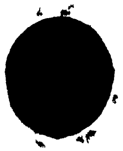
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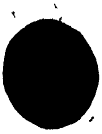
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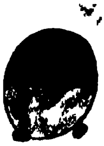
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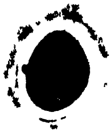
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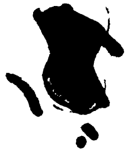
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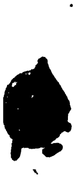
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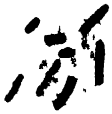
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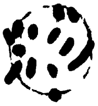
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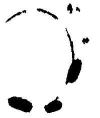
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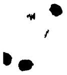
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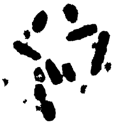
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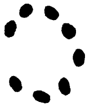
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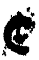
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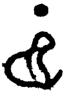
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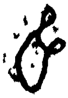
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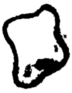
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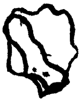
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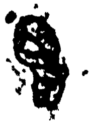
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MITOCHONDRIA IN LEISHMANIA BRASILIENSIS VIANNA, 1911

BY

DAVID CAUSEY

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CONTENTS

	PAGE
Introduction	19
Technique	20
Morphology of the motile phase	21
Mitochondria in the motile phase	21
Summary	24
Literature cited	25
Explanation of plate	28

INTRODUCTION

The presence of mitochondria in flagellates has been reported by a number of observers, Fauré-Fremiet (1910), Alexeieff (1916, 1917), Shipley (1916), and Hogue (1922). Fauré-Fremiet refers briefly to their presence in *Cryptomonas* and in *Chilomonas paramoecium*. Alexeieff reports very briefly the presence of mitochondria in a number of intestinal flagellates: *Giardia anadenalis*, *G. muris*, *G. agilis*, *Trichomonas augusta*, *T. batrachiorum*, *T. muris*, *Protrichomonas legeri*, *Hexamastix termitis*, *Octomastix parva*, *O. motellae*, *Octomitus intestinalis*, *Trichonympha agilis*, *Eutrichomastix lacertae*, *E. motellae*, and *Tetramastix bufonis*. Shipley reported the presence of mitochondria in *Trypanosoma lewisi*, particularly as demonstrated by Janus green in the living organism. Hogue similarly reported their presence in *Trichomonas hominis*. With the exception of Alexeieff, practically no investigator has done more than to report their presence. Alexeieff has considered their functional significance, especially that of the parabasal body, which he considers as a derivative of mitochondria. Shipley found that the mitochondria present in *T. lewisi* did not take part in the motor activities. The presence of mitochondria in the genus *Leishmania* does not appear to have been previously reported.

The material used for this study was obtained from Mrs. E. H. Wagener of this department, to whom I am indebted for both cultures of the organism and for many helpful suggestions in handling the same. This work has been done under the direction of Dr. C. A. Kofoid, to whom I am indebted for suggestions and criticisms. The *Leishmania brasiliensis* used is a culture originally isolated by Dr. H. Noguchi of the Rockefeller Institute for Medical Research, and was obtained in southeastern Brazil. It has been in culture for about two and a half years. Morphologically the strain appears typical, but Mrs. Wagener informs me that attempts to demonstrate its virulence on laboratory animals have so far been unsuccessful. In view of the profound physiological changes which follow the long continued culture of any parasitic form, it is desirable to emphasize the fact that the following results have been obtained on culture material alone. Material from actual cases of leishmaniosis is not available, so that it has not been possible to test the results by observations on such organisms.

TECHNIQUE

The material obtained from cultures was mixed with either physiological saline solution or with the water of condensation itself, and the resulting suspension spread on slides coated with albumin. The latter gave the better results, as vacuolation occurred with the physiological saline solution. The smears were fixed in osmic acid vapor and stained with Regaud's modified haematoxylin as previously reported (Causey, 1925). For a study of the living flagellates Janus green B in physiological saline solution was used to demonstrate the mitochondria and parabasal body, and both Sudan III and Scharlack R were used to demonstrate the fat bodies. The flagellates stain very slowly with the haematoxylin, which is contrary to my experience with other Protozoa; some two or three days in the stain are necessary to stain them sufficiently. Destaining is a matter of seconds, and when properly accomplished the flagellates show the cytoplasmic inclusions as intensely black bodies in a very faintly stained cytoplasm. The Bensley-Cowdry acid fuchsin and methyl green method indifferent results, due to the slow staining.

MORPHOLOGY OF THE MOTILE PHASE

The morphology and neuromotor system of *Leishmania brasiliensis* has been described by Kofoid and Hall (1925). With the technique used here the neuromotor system is not brought out clearly in all organisms on the same slide, and in no case with the same clearness as was obtained with their methods. When visible in the preparations the neuromotor system agrees with their account (pl. 4, figs. 3, 5, 6, 7, 8, 13, 19, 20). The nucleus is difficult to make out after osmic acid fixation, the membrane being very faintly stained and sometimes invisible, while the karyosome is quite black. The parabasal body at times approaches the mitochondria in size and shape so that at times it is difficult to distinguish between them.

The forms of *Leishmania brasiliensis* obtained in culture vary from plasmodial masses containing one or more nuclei and parabasal bodies (fig. 1) to well defined rounded bodies, non-flagellated (figs. 2, 3) or flagellated (figs. 4–7); and from these to the typical slender herpetomonad forms (figs. 12, 13), with numerous transitional forms. In addition to these motile phases occasional minute spherical bodies are found which appear to be cysts. They are surrounded by a definite wall and contain a small mass of cytoplasm in which a nucleus and parabasal body are to be found, together with a reduced number of mitrochondria (fig. 14).

MITOCHONDRIA IN THE MOTILE PHASE

The mitochondria present in *Leishmania brasiliensis* are usually spherical, and appear to be definitely fixed in number. Inspection of slides containing hundreds to the field shows that the number usually present, even in such different forms as the plasmodium (fig. 1), round forms (figs. 3, 4), and herpetomonad forms (fig. 12), is eight—occasionally six. In the herpetomonad forms, as will be discussed later on, two of the mitochondria are transformed into fat bodies.

The presence of a definite number of mitochondria in so small an organism is significant. In larger organisms, e.g., ***Endamoeba gingivalis,*** the organism itself varies so greatly in size in its different phases that it is not feasible to attempt to establish such a relationship. In metazoan tissues, sectioned at a definite thickness, it has been possible to count the number per unit volume of cytoplasm and establish a quantitative relationship for such tissues (Thurlow, 1917). It appears probable that there is a quantitative relation between the mass of cytoplasm and the number of mitochondria present, recalling the nucleo-cytoplasmic ratio. In the cases of apparent departure from the usual number the following possibilities are to be considered: Our present methods of technique for the demonstration of mitochondria are not adequate to demonstrate always all the mitochondria. The utter failure of a reliable technique for such work is not so rare an occurrence as one would wish, and even in the best of preparations the results are never uniform. Organisms side by side on the same slide vary considerably. The cause for this is at present very obscure, but there is as much reason to consider the results as due to a difference in the physiological state of the organism as to faulty technique. It has been shown that in *E. gingivalis* (Causey, 1925) the mitochondria arise *de novo,* and it is probable that in certain organisms the full number has not been established. In other cases it is clear that the missing mitochondria have been transformed into other substances. One of the few facts that investigators of such inclusions agree upon is the transformation of mitochondria into fat bodies (Cowdry, 1924). Such a condition is found in *L. brasiliensis* (figs. 8, 13, 17) where one or two of the missing mitochondria are represented by the same number of fat bodies. When fat bodies are present the number varies from one to three (figs. 9, 10, 13); occasionally only fat bodies are present (fig. 11). The fat body may be in a distinct vacuole (fig. 7) but usually this cannot be determined.

During the division of the organism the mitochondria elongate and become rod shaped. The number present in the dividing organism is variable, there being an increase in number in the early stages following division of the nucleus but not before (figs. 17, 18), after which the number is again reduced (figs. 19, 20). In late stages of division the mitochondria are much reduced in number, while the number of fat bodies present is high (fig. 21). It is evident from a comparison of the neuromotor system and the mitochondria during cell division that there is no direct relationship. The mitochondria appear to be divided approximately equally between the daughter cells, and before division of the cytoplasm has occurred the mitochondria have been transformed into fat bodies. Under such conditions it seems probable that the daughter organisms develop the mitochondria anew until the necessary ratio between the mitochondria and the cytoplasm has been

Causey (1925) has shown that the morphology of the mitochondria in *E. gingivalis* appears to be associated with the metabolic activities of the cell. During the anabolic activities present in the immediate vicinity of the food vacuoles the mitochondria were always rod shaped, while during catabolic activities such as amoeboid movement the mitochondria were spherical. This appears to be true for *L. brasiliensis.* Metabolic activities in a flagellate parasite in the blood of a vertebrate are largely catabolic. The food utilized is derived directly from the surrounding medium, the blood, which must be considered the richest supply of ready-made food available to a living organism. The mitochondria grow readily in culture made of blood serum, and so far as is known the absorbed serum requires no additional changes. No vacuolar digestion has ever been observed. The habitat, a viscous medium, requires a great energy change to permit movement. In active organisms in such a medium spherical mitochondria are found. When cellular division occurs, however, there is considerable anabolic activity. Growth occurs, and division of the neuromotor system accompanies this growth, so that when the division has been completed the daughter organisms are as large as the original cell (fig. 21). Under such anabolic conditions the rod-shaped mitochondria are found. In the final stages of division, after the initial growth has occurred, the spherical mitochondria are found and are gradually replaced by fat bodies (figs. 19–21). In the cyst, where metabolic activities are at a low ebb, the number of mitochondria is reduced and the mitochondria are spherical in form (fig. 14).

Janicki (1915) and Swezy (1916) have pointed out the apparent relationship between the parabasal body and the mitochondria. Shipley (1916) reported that in *T. lewisi* the parabasal body stained with Janus green. The most suggestive statement regarding the function of the parabasal body in flagellates is that of Kofoid and Swezy (1920), that it is "a reserve body connected with metabolism consequent upon the motor functions of the neuromotor system." Alexeieff (1917) considers the parabasal body as of mitochondrial origin. According to him the mitochondria secrete glycogen which is utilized by the motor and metabolic activities in the organism. Boeck (1919) has shown that the parabasal body in *Giardia microti* secretes glycogen.

The only difference observable between the parabasal body and the mitochondria in *L. brasiliensis,* aside from its larger size, is its resistance to acetic acid. It stains with Janus green in the living organism as readily as the mitochondria, and stains equally well in fixed material. That mitochondria vary in their resistance to acetic acid has been reported by various investigators (Regaud, 1910; Nicholson, 1916). Traces of them, poorly fixed, are to be found in *L. brasiliensis* after fixation in Schaudinn's fluid. Although irregular in shape they stain intensely. It seems probable that many, if not all the statements regarding the so-called chromidia in the Protozoa are to be regarded as of this nature (Gatenby, 1919; Kofoid, 1921). The parabasal body in *L. brasiliensis* is often surrounded by a lightly staining area which appears identical with the region in *Giardia microti* in which Boeck (1919) was able to demonstrate glycogen. The minute size of *L. brasiliensis* makes such a test very difficult. Because of the similarity in staining reactions it appears evident that the parabasal body is closely allied chemically with the mitochondria, and Alexeieff's conclusion that it is of mitochondrial origin seems probable.

SUMMARY

1. The mitochondria present in *Leishmania brasiliensis* are usually eight in number, indicating a constant ratio between the volume of cytoplasm and the number of mitochondria.

2. In the non-dividing organism the mitochondria are spherical in form, which is correlated with the catabolic activities of the organism.

3. In the dividing organism the mitochondria are rod shaped in form, a fact which is correlated with the anabolic activities in the organism.

4. The similarity of the parabasal body and the mitochondria in staining reactions indicates that the parabasal body is a derivative of mitochondria.

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Transmitted May 1, 1925.

EXPLANATION OF PLATE

All figures are of *Leishmania brasiliensis*, fixed in vapor of osmic acid and stained in modified haematoxylin. × 3400.

PLATE 4

Fig. 1. Plasmodial phase, beginning to round up.

Fig. 2. Rounded phase with no trace of flagellum. Note small number of mitochondria.

Fig. 3. Rounded phase with intracytoplasmic flagellum. Fat body in vacuole, typical number of mitochondria.

Fig. 4. Rounded phase with typical number of mitochondria and rod-shaped parabasal.

Fig. 5. Rounded phase with typical number of mitochondria and well defined neuromotor system.

Fig. 6. Rounded phase, with neuromotor system and transitional stages from mitochondria to fat bodies.

Fig. 7. Rounded phase with reduced number of mitochondria and fat body in vacuole.

Fig. 8. Transitional stage, with reduced number of mitochondria and four fat bodies. The termination of the fibrils (paradesmose remnants?) posteriorly at fat bodies appears to be accidental.

Fig. 9. Rounded phase with reduced number of mitochondria and fat body.

Figs. 10, 11. Rounded phases with mitochondria nearly and completely replaced by fat bodies.

Fig. 12. Herpetomonas phase with reduced number of mitochondria. Two mitochondria are apparently joined by fibrils.

Fig. 13. Typcal herpetomonas phase. Nucleus has chromatin concentrated on one side of nuclear membrane. Two mitochondria have been transformed into fat bodies.

Fig. 14. Cyst stage. Note reduced number of mitochondria.

Fig. 15. Division stage with two nuclei and rod-shaped mitochondria.

Fig. 16. Moribund form with abnormal cytoplasm and mitochondria.

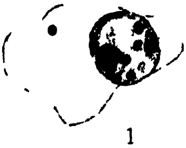
Fig. 17. Early division stage.

Fig. 18. Division stage with two nuclei and twice the number of mitochondria present in non-dividing organism. The mitochondria are rod-shaped.

Fig. 19. Dividing stage with spherical mitochondria. This is interpreted as a later stage than the preceding figure.

Fig. 20. Same as figure 19, but with beginning of transformation of mitochondria into fat bodies.

Fig. 21. Late stage in division. Cytoplasm beginning to divide, the mitochondria reduced in number, and with two large fat bodies in each half. Compare with figure 11.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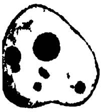
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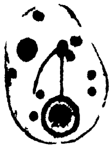
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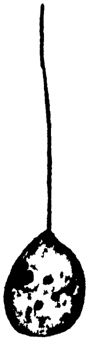
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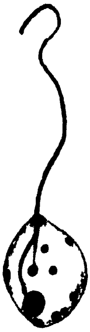
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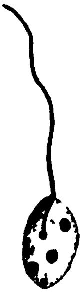
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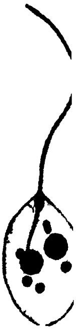

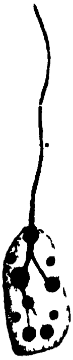
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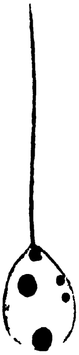
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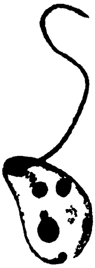
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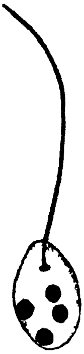
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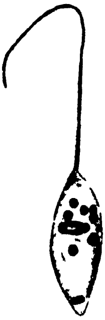
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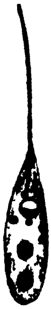
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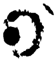
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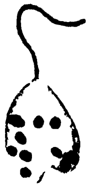
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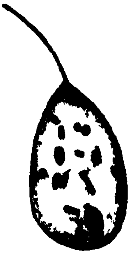
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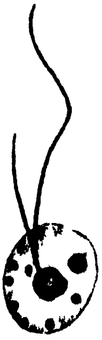
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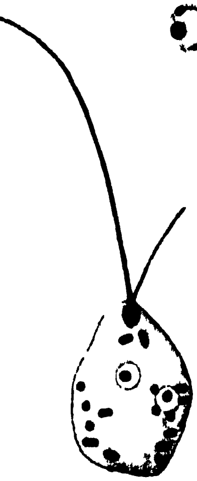
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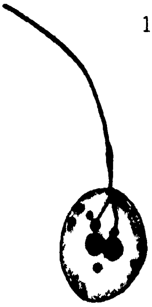
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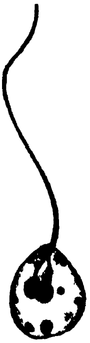
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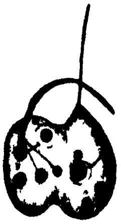
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MITOSIS IN CERATIUM HIRUNDINELLA O. F. M., WITH NOTES ON NUCLEAR PHENOMENA IN ENCYSTED FORMS AND THE QUESTION OF SEXUAL REPRODUCTION

BY

RICHARD P. HALL

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CONTENTS

	PAGE
Introductory and historical	29
Acknowledgments	30
Material and technique	31
Neuromotor system	32
Cytoplasmic inclusions	32
Interphase	33
Prophase	34
Karyosomes	35
Metaphase	37
Anaphase and telophase	41
Nucleus in encysted forms	41
Discussion	44
Neuromotor system	44
Mitosis	44
Amitosis	47
Encystment and sexual phenomena	47
Summary	51
Literature cited	52
Explanation of plates	56

INTRODUCTORY AND HISTORICAL

This paper is the third in a series of investigations undertaken with the purpose of determining, if possible, any fundamental relationships in mitosis at binary fission that may exist among several different groups of the Mastigophora. In the two earlier papers, it has been shown that in the euglenoid, *Menoidium incurvum* (Hall, 1923), and the primitive dinoflagellate, *Oxyrrhis marina* (Hall, 1925), there are several points of similarity. (1) There is present in both flagellates a typical *neuromotor system*, consisting of flagella, flagellar rhizoplasts, and extranculear centrosome. (2) A *paradesmose* connects the daughter centrosomes as they draw apart after division at the beginning of binary fission. (3) A true mitosis occurs—that is, the chromosomes are split longitudinally, and the daughter chromosomes are separated as whole chromosomes by the transverse bisection of a metaphase plate, established previously by the unfolding of the daughter elements of the splitting chromosomes to form a cylindrical belt. (4) The nucleus contains an *endosome,* which elongates and finally constricts into two parts in mitosis, behaving as a sort of central spindle for the chromosome belt.

The present investigation of *Ceratium hirundinella* was begun in the anticipation that some knowledge might be gained in regard to the following: (1) presence or absence of a neuromotor system, and the nature of such a system, if present; (2) method of splitting of the chromosomes, and the relation of this process to the formation of the equatorial plate; (3) the significance of encystment, especially in regard to its possible relation to sexual reproduction.

The division of *Ceratium hirundinella* has been described by several of the earlier writers, including Blanc (1885) and Lauterborn (1895), and the general morphology and the gross features of mitosis and binary fission in this flagellate have been well known for a number of years. Blanc (1885) was the first to publish an account of nuclear division in *Ceratium:*

> . . . . je suis affirmer qu'en tous cas, le *Ceratium hirundinella* se reproduit par division, après division prealable de son noyau.

Beyond this one fact that nuclear division precedes fission of the organism, Blanc's paper contributed little of value to the knowledge cf mitosis in *Ceratium;* his few figures of dividing nuclei show no chromosomes, although the presence of chromomeres is indicated. Lauterborn's (1895) later account of nuclear division and binary fission in this species contains an excellent description of the general features of mitosis, although he did not *describe* splitting of the chromosomes.

ACKNOWLEDGMENTS

The writer wishes to thank Professor Charles A. Kofoid for his kindness in lending the material upon which this study is based, and for his many helpful criticisms during the course of the investigation; and also, to express his indebtedness to Dr. Olive Swezy for her suggestions in regard to preparation of the plates. Acknowledgment is also made to the Elizabeth Thompson Science Fund for a grant in aid which made possible the collection of the extensive series of plankton samples used in this research and the preparation of the slides.

MATERIAL AND TECHNIQUE

The material for this study of *Ceratium hirundinella* was obtained during August, 1922, from the Lake Pillarcitos reservoir of the Spring Valley Water Company of San Francisco. Plankton-net catches were made every hour throughout a period of thirty-six hours. Each catch was killed at once in Schaudinn's fluid, and then partially dehydrated and stored in alcohol. One portion of each catch was later stained in bulk in iron-alum haematoxylin and mounted in balsam. A second part was imbedded in paraffine and sectioned at 7.5μ; the sections, also, were stained in iron-alum haematoxylin.

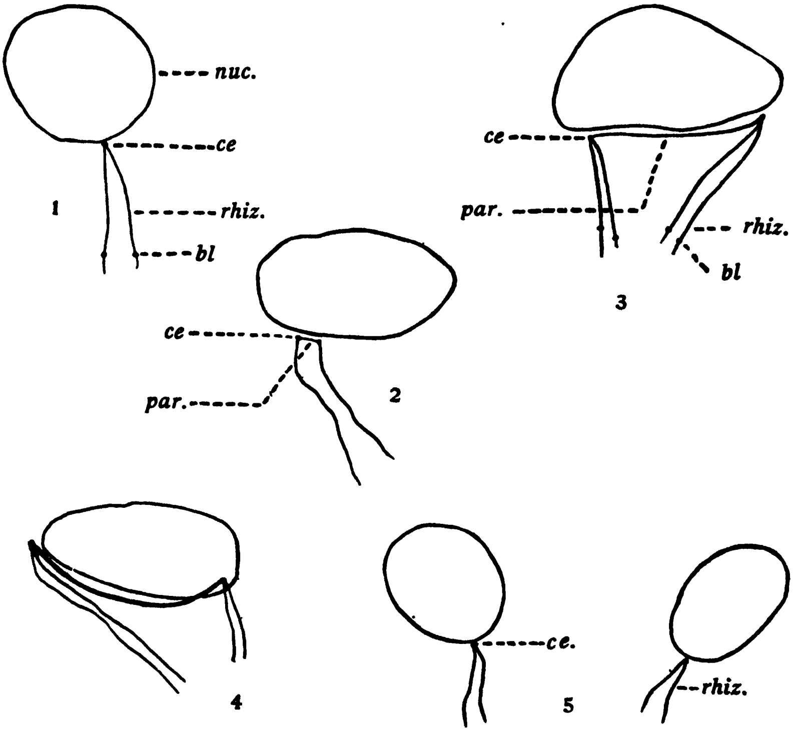

Fig. A. Neuromotor system of *Ceratium hirundinella;* diagrammatic camera lucida drawings from material stained in Bordeaux red followed by iron-alum haematoxylin. (1) Interphase, centrosome undivided; (2) prophase, two daughter centrosomes connected by the paradesmose; (3) early metaphase; (4) metaphase, equatorial plate stage; (5) telophase, daughter nuclei completely separated; *bl.*, blepharoplast; *ce.*, extranuclear centrosome; *nuc.*, nucleus; *par.*, paradesmose; *rhiz.*, flagellar rhizoplast. × 1200.

In addition, preserved material was stained as follows: (*a*) Bordeaux red followed by iron-alum haematoxylin, as a stain for demonstration of the neuromotor system; (*b*) borax-carmine; (*c*) iron-alum haematoxylin followed by eosin; (*d*) eosin followed by methylene blue. None of this material was sectioned.

NEUROMOTOR SYSTEM

The neuromotor system (fig. A) in *Ceratium hirundinella* is homologous, in all essential respects, with that of *Oxyrrhis marina* (Hall, 1925). The two flagella—*longitudinal and transverse*—lie in the *sulcus* and *girdle,* respectively, and, passing through the *flagellar pores,* end in *blepharoplasts* just inside the cytoplasm. From each blepharoplast a fine *flagellar rhizoplast* extends to a granule, the *extranuclear centrosome,* adjacent to the nucleus.

The behavior of the neuromotor system during mitosis is also similar in the two flagellates, *Oxyrrhis* and *Ceratium.* At the beginning of binary fission in *Ceratium* the extranuclear centrosome divides, and the two daughter centrosomes, to each of which is connected a flagellar rhizoplast, begin to draw apart. As the daughter centrosomes become separated, they remain joined together for a time by a *paradesmose,* similar to that found in *Oxyrrhis.* Later on, at about the time splitting of the chromosomes begins, two new flagella, completing the two pairs, appear (pl. 6, fig. 15), presumably by outgrowth as in many other flagellates. After the division of the nucleus, a daughter centrosome, with its attached pair of flagellar rhizoplasts, passes along with each daughter nucleus into one of the schizonts before the completion of fission.

CYTOPLASMIC INCLUSIONS

Besides the neuromotor system, densely staining spherical bodies—probably masses of reserve food or waste materials—are frequently to be observed in the cytoplasm. These structures are often contiguous to the nucleus (pl. 5, fig. 9), but they sometimes lie at quite a distance from it. In one instance (pl. 5, fig. 14) a large spherical inclusion, almost equal to the nucleus in size and homogeneous except for a dumbbell-shaped structure near one edge, lies next to the nucleus, indenting its surface. These inclusions resemble in appearance the "Nebenkörperchen" described by Borgert (1910 *a*) in *Ceratium tripos*. Similar bodies have been observed in the cytoplasm of *Ceratium furca* and *Ceratium fusus* by Pouchet (1883), who offered several suggestions as to their significance, but concluded that "leur nature paraît incertaine."

INTERPHASE

The nucleus of the interphase, in *Ceratium hirundinella,* is considered by Entz (1921) to be characterized by chromatin in the form of disconnected granules, rather than as chromomeres combined into distinct chromosomes. This interpretation, however, is contrary to that of Borgert (1910 *a*) for *Ceratium tripos.* Entz, after stating his interpretation, adds that "besonders schön läßt diese Kügelchenstruktur der Kerne von *Ceratium hirundinella* an Examplaren beobachten, welche, *an den Objektträger angetroknet,* sich nach Giemsa färben ließen." Observations based on such material as he has used are subject to the objection that the dry-film method is not the best method by far for staining the nucleus of the Protozoa, and it seems inadvisable to base a conception of nuclear structure primarily on the study of such preparations.

Lauterborn (1895) is of the opinion that the resting nucleus of *Ceratium* contains granules of chromatin imbedded in the nodes of a linin network; his resting nucleus, however, is considered by Entz to be merely a stage in the early prophase.

Jollos (1910), corroborating Lauterborn's description, finds that the resting nucleus in three marine species of *Ceratium* (*C. tripos, C. fusus,* and *C. furca*) is similar to that described by the latter author for *Ceratium hirundinella.*

Borgert (1910 *a*) concludes that the resting nucleus of *Ceratium tripos* does not contain separated chromomeres, and that such an interpretation of the organization by other authors is due to the observation of optical sections of granular chromosomes, the state of chromatin organization found normally in the nucleus of this species.

So far as can be determined in my preparations, the earliest stage of mitosis shows short chains of chromomeres in the nucleus (pl. 5, figs. 1–3). The presence of a resting nucleus, such as that described by Entz, in which only separate chromomeres are visible, has not been determined in my material. The organization of the nucleus in the early prophase, however, as discussed below, indicates that a very simple organization of the chromatin is to be expected in the nucleus of the interphase, and it is possible that Entz's "resting" nuclei are merely not present in my material. But the spacing of our collections over a period of thirty-six hours renders this improbable.

PROPHASE

The prophase, as outlined by Entz (1921), begins as the separate chromatin granules of the resting nucleus become grouped into parallel rows; these rows of granules, however, *are not considered to be the actual chromosomes* characteristic of later stages. A vacuole next appears in each chromatin granule. These vacuoles enlarge so that their walls come in contact with one another to form a *honeycomb network,* with small chromatin granules lying in the node points—this stage of the prophase is considered by Entz to be homologous with the resting nucleus described by Lauterborn (1895). The chromatin elements, presumably derived from the walls of the vacuolated chromomeres, now fuse into threads, forming a so-called *'thread-skein.'* In the following stages, each thread-skein breaks up into longitudinally split chromosome-segments (the "Chromatinglieder"). These "Chromatinglieder" then *"verbinden sich zu Chromosomen und bilden die Äquatorialplatte mit der Länge nach gespaltenen Chromosomen."*

What Entz has described is essentially a longitudinal division of the chromosomes by some sort of a process of vacuolation, a phenomenon fairly common in the longitudinal splitting of both metazoan and metaphytan chromosomes (Sharp, 1921). These stages included in Entz's outline of chromosome behavior, however, are not figured in his plates of *Ceratium;* they are merely indicated in several schematic text figures, and it is unfortunate that he has not published careful figures of some of the critical stages in the process described.

In my material the chromosomes of the early prophase (pl. 5, figs. 1–3) are few in number, short, and are scattered about loosely in the nucleus, which, otherwise, is stained homogeneously. These nuclei are comparable to the 'spireme stage' figured by Calkins (1899, pl. 40, figs. 6, 7) for *Noctiluca milaris.* In the nuclei of *Ceratium hirundinella* (stained in iron-alum haematoxylin), however, there seems to be none of the 'oxychromatin' granules shown in the figures of Calkins. It is probable that, in these early prophase stages, the chromosomes are in the process of being condensed from a more diffuse state of organization, whatever it may be, which has existed in the nucleus of the interphase.

In later stages of the prophase (pl. 5, figs. 4–13), the chromosomes have become much longer, and are now coiled about in the nucleus. The differences in appearance between the early and later stages are quite striking; in the former (pl. 5, figs. 1–3), the chromosomes are short, and they by no means fill up the space within the nucleus—this is especially evident in figure 1 (pl. 5); in the later stages (pl. 5, figs. 4–13), the chromosomes are much longer, as determined by tracing them through a number of focal planes, and the space within the nuclear membrane is practically filled with chromosomes.

In some cases (pl. 5, fig. 10), there is a suggestion of a radial polarization of the chromosomes in the later prophase, resembling that observed in *Oxyrrhis marina* (Hall, 1925).

KARYOSOMES

One or more karyosomes are usually to be seen in the nucleus during the prophase; in my material from one to four, ranging in diameter from 1 to 3μ, have been observed in the nuclei of vegetative forms. They are usually spherical, but are sometimes ovoid or even elongated; they are also homogeneously stained as a rule, but a number have been found to contain a faintly stained central region (pl. 5, figs. 8, 12; pl. 6, fig. 20), possibly a vacuole.

Blanc (1885) believed that the nucleus of this species of *Ceratium* contains, typically, a single karyosome which divides regularly at mitosis. A karyosome of this type (*endosome*) is found in *Oxyrrhis* and the euglenoids, but later workers have shown that there is no such regularity in the behavior of the karyosomes in *Ceratium*. In *Ceratium hirundinella,* these bodies seem to disappear gradually during the late prophase and early metaphase. Elongation of the karyosomes may occur, but a division has never been recorded. This is decidedly in contrast to the condition in *Oxyrrhis marina* (Hall, 1925) and *Gymnodinium* (*Gyrodinium*) *fucorum* (Jollos, 1910), in which a division of the endosome occurs during each nuclear division.

As to the morphological relationships of the karyosomes, Entz (1921) has found instances in which these bodies are connected by fibrils ("desmoses") to single chromatin granules; structures something like these were observed by Jollos (1910) in *Ceratium tripos.* These fibrils resemble similar ones (pl. 5, figs. 11, 12) observed by the writer in *Ceratium hirundinella,* extending, in one case, from the karyosome to a point outside the nucleus. Such scattered observations suggest the possibility of the presence of an intranuclear rhizoplast in connection with one of the karyosomes, like that found in *Oxyrrhis marina,* but there is as yet little reliable evidence that such a fibril exists in *Ceratium hirundinella.*

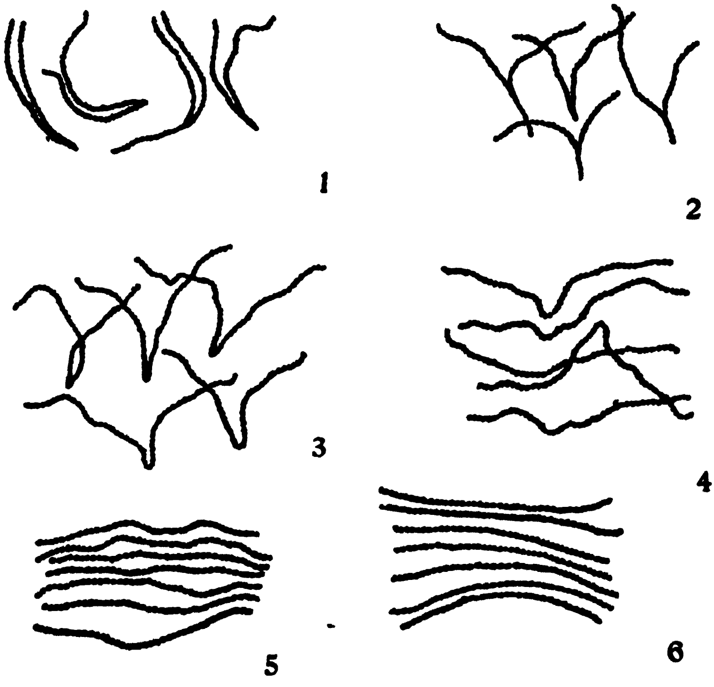

Fig. B. Tracings of individual chromosomes from nuclei figured in plates 6–9; (1–3) late prophase and early metaphase; (4–6) metaphase. × 1600.

Jollos (1910), in addition, has figured a granule resembling a centriole in one of the karyosomes of *Ceratium tripos;* he is, however, somewhat uncertain as to whether or not it bears any relation to the initiation of mitosis.

METAPHASE

In the early metaphase, longitudinal splitting of the chromosomes begins. There is no evidence, in my material, that this splitting involves a vacuolation of the individual chromomeres, as described by Entz (1921). Splitting appears to be initiated at one end of each chromosome, and to be extended gradually toward the other. Figure B (1, 2) and figures 15–19 (pl. 6) show a number of Y-shaped chromosomes in which splitting is not yet completed. Figure 18 (pl. 6) represents a nucleus containing a number of double and Y-shaped chromosomes, formed presumably by longitudinal splitting; this stage resembles similar nuclei figured by Entz (1921, pl. 13, figs. 8, 9). As splitting proceeds, the freed ends of the daughter chromosomes appear to move apart toward opposite poles of the nucleus (fig. B, 1–3; pl. 6, figs. 17, 19–22), and, before the completion of the splitting, the ends of these daughter chromosomes have often moved quite a distance apart, as indicated in figure B, 2 (also pl. 6, figs. 15–17). While splitting is being completed, unfolding of the daughter chromosomes is continued (fig. B, 3; pl. 6, figs. 19, 21); the result is a nucleus containing irregular V-shaped and U-shaped chromosomes, the arms of which extend toward opposite poles of the nucleus. In figure 22 (pl. 6), most of the chromosomes have already unfolded, and the arms of the V's show indications of the parallel arrangement which is to appear in later stages.

Entz (1921) does not describe stages which show the splitting and unfolding processes described above, but he does figure chromosomes which strongly suggest the unipolar splitting apparent in my preparations. Figure C, 1, shows a group of chromosomes picked out of two nuclei (Entz, 1921, pl. 13, figs. 8, 9) which Entz has interpreted as indicating an end-to-end fusion of the "Chromatinglieder" (longitudinally split chromosome-segments). In these nuclei, there are chromosomes in which unipolar splitting seems to have just begun; in other chromosomes, splitting appears to be almost completed; and, in a few, an unfolding process is under way.

Lauterborn (1895) also figures chromosomes (in his pl. 12, figs. 9, 10) which may be interpreted as showing various stages of unipolar splitting with consequent unfolding. In figure C, 2, there are shown two chromosomes in which splitting is about half completed; in the others, splitting is practically completed, and a few have begun to unfold. Figure C, 3, represents a similar group of chromosomes in which splitting appears to be completed, and unfolding is under way.

Borget (1910 *a*, pl. 2, figs. 11, 16, 17) likewise seems to have observed unipolar splitting of the chromosomes, although he did not describe it as such. In a nucleus of *Ceratium tripos* (fig. C, 4), he shows chromosomes which appear to be in early and late stages of unipolar splitting. In his figures of *Ceratium fusus*, a number of the chromosomes appear to be in various stages of unfolding after the completion of unipolar splitting (fig. C, 5). In addition, the derivation of his figure 18 (pl. 2) from figures 16 and 17 might readily be explained on the basis of unipolar splitting, followed by an unfolding of the separating daughter chromosomes; although his figure 17 is

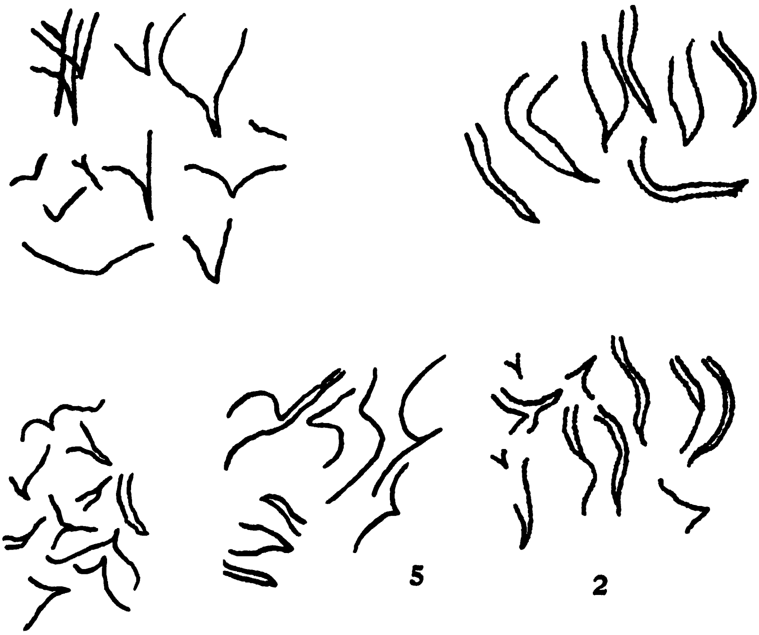

Fig. C. (1) A group of chromosomes picked from nuclei figured by Entz (1921, pl. 13, figs. 8, 9) as stages depicting an end-to-end fusion of longitudinally split chromosome-segments. Note appearance of longitudinal splitting and unfolding. (2, 3) Two groups of chromosomes from Lauterborn's (1895, pl. 12, figs. 9, 10) figures of *Ceratium hirundinella;* the linin threads joining the chromosomes in his figures are omitted. (4, 5), Two groups of chromosomes from nuclei figured by Borgert (1910 *a*, pl. 2, figs. 11, 16, 17), the first group from *Ceratium tripos* and the second from *Ceratium fusus.* All chromosomes in this figure were traced from the original figures of the authors.

very small, it shows several chromosomes which resemble those observed in Ceratium hirundinella in later stages of unfolding.

In the later metaphase, the unfolding of the splitting chromosomes is continued (fig. B, 3–4; pl. 6, figs. 21, 22), and the V-shaped pairs gradually become straightened out (fig. B, 4–6) as the ends of the daughter chromosomes reach the poles of the nucleus. In figure 22 (pl. 6) the unfolding process is nearing completion, and the chromosomes are beginning to straighten out. In figure 25 (pl. 6), unfolding is practically completed; while figure 26 represents the completed metaphase 'plate,' in which a distinctly parallel arrangement of the chromosomes is evident.

As may be seen from the foregoing description of the formation of this metaphase plate, the members of each pair of daughter chromosomes are joined only at one end, that lying in the equatorial plane. The daughter chromosomes are finally separated completely in the equatorial plane (pl. 7, fig. 27), and the separation of the two groups of chromosomes is begun. The originally longitudinal splitting of the chromosomes is thus completed as a transverse division of the equatorial plate at the end of the metaphase.

In addition to the interpretation of longitudinal splitting of the chromosome as adhered to in this paper, the appearance of the chromosomes in the metaphase plate might be accounted for in several other ways. In the first place, the chromosomes may be split longitudinally in the early stages of division, or late in the preceding stage. These longitudinally split chromosomes might then become arranged parallel to each other and then divide transversely in the metaphase, so that a *true mitosis* (according to the conception of linear distribution of the elements of the chromosomes) does not occur. Another possibility is that the chromosomes are not divided longitudinally at all, but that they merely become grouped into a bundle (so that the apparently double chromosomes of the prophase might be explained by incidental pairing rather than by splitting), which is divided transversely in the metaphase—another case without true mitosis. In view of the various stages found in my material and in the papers of others (cited above), as well as the fact that true mitosis is known in other Dinoflagellata (*Noctiluca*), it seems that the interpretation advanced in this paper is the more probable one—namely, that longitudinal splitting of the chromosomes does occur, and that the separating daughter chromosomes unfold to form an end-to-end joined pair (in metaphase plate), the members of which are separated finally by a transverse division of the metaphase plate.

According to Entz (1921), the longitudinally split "Chromatinglieder" fuse end-to-end to form long double chromosomes; these then become arranged parallel to each other in the metaphase plate. There is no evidence in my material for such a fusion of split segments of chromosomes. And even on the basis of Entz's paper it might be pointed out that, given an early stage in which the chromosomes are short and a later stage in which the chromosomes are long, it does not follow necessarily that the long chromosomes have arisen by an end-to-end fusion of the shorter ones. Moreover, there is in my material no recognizable paired arrangement of the chromosomes in the late prophase and early metaphase (pl. 6, figs. 19, 21, 22, 25); according to the hypothesis of Entz, the chromosomes of this stage should be double—in my material they are single. Furthermore, Entz has not figured any of the stages in the early metaphase which have been observed in my preparations, and it is in these stages (pl. 6, figs. 15–17, 19–22) that the process of apparent unfolding of the daughter chromosomes is to be observed. To summarize the matter, Entz figures various steps in unipolar splitting, as well as completely split chromosomes (fig. C, 1); but he has not figured the critical stages of the late prophase and early metaphase, in which unfolding of the chromosomes occurs, and *in which there are no paired chromosomes* to be observed in my material—a fact which seems to invalidate his hypothesis of the fusion of double chromosome segments to form the metaphase plate.

Borgert (1910, *a*, *b*, 1911), in *Ceratium tripos* and *Ceratium fusus*, describes essentially the same phenomenon that Entz has proposed for *Ceratium hirundinella*. The chromosomes are split longitudinally in a 'spireme' stage of the prophase, and the double chromosomes (not chromosome segments, in this case) so formed become arranged parallel to each other in the equatorial plate. These double chromosomes are then divided transversely in the metaphase. As pointed out above (fig. C, 4, 5), Borgert also figures chromosomes undergoing unipolar splitting and unfolding; and, like Entz, he has not included figures of the early metaphase. Jollos (1910), who has also studied *Ceratium tripos*, fails to find any indication of a double structure, or paired arrangement, of the chromosomes in the metaphase plate, a point upon which the hypothesis of Borgert and Entz depends.

Schneider (1924), in a brief paper on *Ceratium tripos*, agrees with Borgert's (1910 *a*, *b*; 1911) description of transverse division of the chromosomes in this species.

ANAPHASE AND TELOPHASE

After the separation of the two groups of daughter chromosomes in the metaphase (pl. 7, fig. 27), reorganization of the daughter nuclei begins as they draw apart during the anaphase. At first, the chromosomes retain their parallel arrangement (pl. 7, fig. 28, one of the two nuclei) characteristic of the late metaphase, but they soon begin to lose this, and in the later stages (pl. 7, figs. 29, 30) the chromosomes have begun to show the coiled organization seen in the prophase. In the late prophase (pl. 7, fig. 30), the structure of the nucleus resembles that of the nucleus found in the prophase (pl. 5, figs. 4–13).

Fission is often preceded by the formation of a temporary chain, as described previously by Entz (1924, fig. 3).

THE NUCLEUS IN ENCYSTED FORMS

The cytoplasm of the cyst is often vacuolated (pl. 8, fig. 33; pl. 9, figs. 40–45), but this characteristic vacuolation is often obscured by heavy protoplasmic strands extending throughout the cytoplasm. These cytoplasmic fibrils are much more distinct than similar ones observed in the flagellate stages. In addition, a more heavily stained area of cytoplasm is nearly always present in the region of the nucleus pl. 8, figs. 35–38; pl. 9, figs. 40–45); this dense region often contains a clear elongated area (pl. 8, figs. 37, 38), resembling somewhat the 'amphiaster' of *Noctiluca* (Calkins, 1899).

Entz (1921) describes the resting nucleus of the cyst as containing chromatin in the form of very small spheres, about half the size of the chromomeres in the nucleus of the flagellate stage; in other words, he believes that the organization of the chromatin in the resting nucleus is essentially similar in both flagellate and encysted forms.

The writer has observed similar stages (pl. 9, fig. 39) in which the nucleus of the cyst, in surface view, appeared to contain separate chromomeres only. In addition, in sectioned material stained in iron-alum haematoxylin, distinct chromosomes have been observed (pl. 8, figs. 31–34). The organization of the second type of nuclei is similar to that of the nuclei of flagellate forms during the prophase. In all cases, however, the size of the individual chromomeres has seemed to be somewhat less than that of the chromomeres in flagellates.

The chromosomes of the cyst nucleus seem to be more densely packed together than those in the nucleus of flagellates, and this appearance points to a possibility that they may be more numerous. This condition, with smaller and apparently more numerous chromosomes, suggests (rather remotely, however), that the cyst nucleus may represent a synkaryon formed as a result of conjugation.

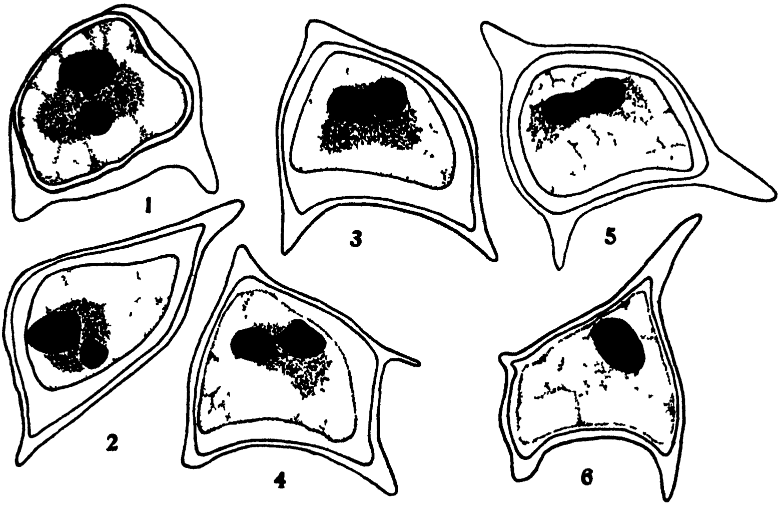

Fig. D. Camera lucida sketches of a series of cysts of *Ceratium hirundinella*; material stained in Bordeaux red followed by iron-alum haematoxylin; nuclei shown in black. (1) Binucleate cyst, nuclei imbedded in dense central mass of cytoplasm; (2) binucleate cyst with unequal nuclei; (3) binucleate cyst with the two nuclei more nearly equal in size and closer together; (4) binucleate cyst with nuclei nearly equal in size; (5) uninucleate cyst with elongated nucleus; (6) uninucleate cyst. × 480.

The shape of the nucleus of the cyst varies. It may be elongated (pl. 8, figs. 34, 37, 38), or even U-shaped (pl. 8, figs. 35, 36), in addition to the more common spherical shape usually observed (pl. 8, figs. 31, 32).

A binucleate condition, apparently, has never before been described in the encysted forms of *Ceratium hirundinella.* Entz (1921), in *Ceratium hirundinella,* finds in "allen Cysten nur einen Kern," and in none of the other papers which have come to the attention of the writer is there any reference to a cyst containing more than one nucleus. A large number of binucleate cysts, however, have been observed in my material (fig. D, 1–4; pl. 9, figs. 39–45). This binucleate condition may have arisen in one of two ways. First, the primary nucleus of the cyst may have undergone nuclear division to form two daughter nuclei. Second, the two nuclei may be contributions from two conjugating individuals, so that the cyst represents a zygote.

The *evidence for nuclear division* in the cyst may be summarized as follows:

(1) Both uninucleate and binucleate stages have been found in my material (pls. 8, 9; fig. D), and nuclear division within the cyst is a common phenomenon in flagellates and in the Protozoa in general.

(2) Distinct chromosomes have been observed in the nucleus of the cyst (pl. 8, figs. 31–34); the organization of these chromosomes suggests that of the prophase nuclei in flagellates.

(3) Elongated nuclei (pl. 8, figs. 34–38) have been observed, and occasionally such nuclei are found to be constricted in a manner suggestive of impending nuclear division.

(4) In binucleate stages, the two nuclei are often found close together, imbedded in a mass of cytoplasm resembling an amphiaster (fig. D, 1–4; pl. 9, figs. 41, 43–45).

The *objections to assuming that nuclear division has occurred* may be listed as follows:

(1) There is no conclusive evidence in my material that nuclear division occurs, since the organization of the chromosomes, as observed so far, is not necessarily indicative of mitosis.

(2) Elongation of the nucleus may be a 'normal' condition not preparatory to division, since the nucleus of the flagellate stage is typically elongated; likewise, the constriction of such nuclei is not necessarily a preliminary step in division.

(3) The close conjunction of the nuclei in binucleate stages may be accounted for by nuclear conjugation, following a process of conjugation in which each conjugant has furnished a nucleus to the cyst; while the uninucleate stage of the cyst may have been produced by the fusion of such 'gamete' nuclei.

The possible relation of these binucleate cysts to a process of sexual reproduction is discussed below.

DISCUSSION

The Neuromotor System

A *neuromotor system* has already been described for one of the dinoflagellates (*Oxyrrhis marina,* Hall, 1925), belonging to the family *Protodiniferidae,* tribe *Thecatoidae* (Kofoid and Swezy, 1921). The demonstration of an homologous system in *Ceratium hirundinella,* which belongs to a widely separated group—Tribe *Peridiniidae* (Kofoid and Swezy, 1921)—suggests that the neuromotor system is a fundamental characteristic of the Dinoflagellata. A neuromotor system has also been demonstrated in the following groups of the Mastigophora: *Polymastigina* (Kofoid and Swezy, 1915 *a, b;* Swezy, 1915 *a, b;* Kofoid and Swezy, 1919 *a;* Cutler, 1919; Rhodes, 1919; Kofoid and Swezy, 1920, 1923), *Distomatina* (Kofoid and Christiansen, 1915 *a, b, c;* Boeck, 1917; Kofoid and Swezy, 1922), *Hypermastigina* (Kofoid and Swezy, 1919 *b, c;* Kirby, 1924), *Euglenoidina* (Baker, 1921; Hall, 1923). Also, fragmentary evidence (Awerinzew, 1906–1907) indicates that a neuromotor system is characteristic also of the *Cryptomonadina.*

The paradesmose, likewise, is a fundamental structure in the groups listed above, and is known to be present in all the groups in which a neuromotor system has been determined definitely. Nägler (1912), in addition, has figured a fibril resembling a paradesmose in one of the Cryptomonadina (*Chilomonas paramecium*).

Mitosis

Nuclear division in the genus *Ceratium* has been a subject of discussion since the appearance of Lauterborn's (1895) paper on *Ceratium hirundinella.* Nuclear division in this species, as outlined by Lauterborn, could not be classified as a true mitosis, since no splitting of the chromosomes is described; instead, the chromosomes are supposed to be bisected transversely during the metaphase plate stage. This scheme would mean that nuclear division involves merely a halving of the chromatin material, without any relation to a quantitative division of the chromosomes—a phenomenon not at all in accord with the modern conception of the behavior of the chromosomes in mitosis.

Jollos (1910) has described nuclear division in three marine species of *Ceratium,* but he fails to find any longitudinal splitting of the chromosomes.

Borgert (1910 *a, b,* 1911) has described, in several papers, his discovery of a "neue Form der Mitose" in *Ceratium tripos.* Longitudinal splitting of the chromosomes was observed, and there is figured a sort of spireme stage ("knauelstadium"), in which longitudinally split chromosomes are visible. In a *true mitosis,* the daughter elements of the longitudinally split chromosomes would be completely separated in the metaphase; in Borgert's scheme of mitosis, the daughter chromosomes formed by splitting remain paired and parallel to each other in the formation of the equatorial plate. Instead of being separated along their longitudinal axes, the daughter chromosomes are divided transversely in the metaphase, so that a half of each, along with the corresponding half of its mate, passes to one of the daughter nuclei. The paired arrangement of these segments is said to be retained during the anaphase and telophase, but to be lost during the reorganization of the daughter nuclei. Splitting of the chromosomes occurs again in the following prophase. This type of nuclear division obviously does not fit into any process of true mitosis.

Entz (1921) likewise discusses a "mitotische" division in *Ceratium hirundinella,* yet his outline of nuclear division is essentially the same as that proposed by Borgert (1910 *a, b,* 1911). During a spireme stage of the prophase, short longitudinally split "Chromatinglieder" are established by an intricate method involving vacuolation of the individual chromomeres; these split chromosome-segments then *fuse end-to-end to form long double chromosomes,* which become grouped parallel to one another in the equatorial plate. A transverse division of these chromosomes next occurs, just as described by Borgert for *Ceratium tripos.* In *Ceratium hirundinella,* as in *Ceratium tripos,* the paired arrangement of the chromosomes is supposed to persist during the anaphase and telophase, and it is suggested that, during the period of reorganization of the daughter nuclei, these paired chromosomes may fuse longitudinally with each other to form the definitive chromosomes of the two schizonts.

Schneider (1924), in a brief paper on *Ceratium tripos,* has described a transverse division of the chromosomes which he believes is identical with that described by Borgert (1910 *a, b,* 1911) in the same species.

From the descriptions of nuclear division presented by the authors mentioned above, it is obvious that a typical mitosis has not been established for any of the species of *Ceratium.* The occurrence of a true mitosis in the Dinoflagellata, however, was established years ago when Calkins (1899) demonstrated the process in *Noctiluca milaris.* This author has shown indisputably that, in *Noctiluca,* longitudinal splitting of the chromosomes is begun at their ends nearest the centrosphere, and is carried progressively toward the distal ends. Later the proximal ends of the separating daughter chromosome begin to move apart and to migrate toward opposite poles of the amphiaster (formed by division of the centrosphere). Final separation of the daughter chromosomes takes place during the metaphase, along the equatorial plane of the metaphase plate. In this case, *entire daughter chromosomes are separated by a transverse division of the metaphase plate, after a previous longitudinal splitting of the chromosomes;* there is no transverse division of the chromosomes, such as that described by Borgert and Entz in *Ceratium.*

In *Oxyrrhis marina* (Hall, 1925), one of the more primitive dinoflagellates, it has been found that nuclear division is similar to the process described by Calkins in *Noctiluca.* The chromosomes appear to be split longitudinally, while the daughter chromosomes unfold, as in *Noctiluca,* to form an equatorial plate; the final separation of the daughter chromosomes is completed during the metaphase, when the equatorial plate divides into two halves.

In *Ceratium hirundinella* also, according to our findings, nuclear division is similar to that in *Noctiluca.* In the material studied, longitudinal splitting of the chromosomes occurs, as is also described by Entz (1921). However, in stages seemingily overlooked by Entz, it appears that the daughter chromosomes do not remain paired, but separate by an unfolding process, which results in the formation of the equatorial plate (fig. B). This equatorial plate is composed then, not of paired daughter chromosomes arranged parallel to each other as described by Entz, but of the unfolded daughter chromosomes still joined at their ends which lie in the equatorial plane (see fig. B). The transverse division in the equatorial plane merely completes the separation of these daughter chromosomes, and *does not effect the transverse division of whole chromosomes.* It has been pointed out above (fig. C) that Lauterborn, Borgert, and Entz have all figured chromosomes which may be interpreted as showing various stages of unipolar splitting and unfolding, and which support my own observations on *Ceratium hirundinella.* In short, the evidence indicates that a true mitosis occurs in *Ceratium hirundinella,* just as in *Noctiluca milaris.*

AMITOSIS

Amitosis, or "direkte Kernhalbierung," has been described as an occasional occurrence in *Ceratium tripos* by Borgert (1910 *a*), who offers as evidence several figures of elongated, constricted or bilobed nuclei. He recognizes two types of amitosis, one involving "verschiedene Modalitäten der Querteilung," the other a "Längsteilung." Since the writer has never examined preparations of *Ceratium tripos,* it is possible only to criticize Borgert's evidence, which seems inadequate. It might be stated that the nucleus of *Ceratium hirundinella* is also far from constant in shape; many variations, including bilobed forms, elongated types, and even U-shaped nuclei have been observed. Many of these 'abnormal' nuclei resemble the figures offered by Borgert as stages in amitosis. There is no evidence that such misshapen nuclei proceed to an amitotic division in *Ceratium hirundinella,* and Borgert has failed to prove that a division of this sort occurs in *Ceratium tripos.* In his plate 3, figure 29, he shows what is assumed to be the completion of a direct division by modified "Querteilung"; the stage appears to be nothing more than a telophase at a lâter stage than his figure 23. Nor in his scheme of "Längsteilung" has he presented any conclusive evidence that the nuclei divide by this method; his last figure of this series apparently represents merely a deeply constricted nucleus, resembling those observed occasionally in my preparations of *Ceratium hirundinella.*

ENCYSTMENT AND SEXUAL PHENOMENA

The question of the relation of encystment to the life-history of the Protozoa has long been an interesting problem to protozoologists, and has offered a subject for much speculation as well as careful investigation. Encystment has been said, in some cases, to follow sexual phenomena, so that the encysted individual represents a zygote. In other cases, the cyst is believed to be formed in response to the advent of unfavorable environmental conditions. In still other instances, it is clear that encystment follows the ingestion of food, as the result of a need for "a quiescent period for its assimilation" (Kofoid and

Swezy, 1921). And finally, the cyst may be formed for "protection during the non-motile stage of binary and multiple fission" (Kofoid and Swezy, 1921) in many forms.

In the dinoflagellates as a whole, 'feeding' cysts and 'reproductive' cysts are commonly observed. In addition to these types, Zederbauer (1904) has described a "Kopulation" process in *Ceratium hirundinella,* preceding the formation of a "Zygospore," which "werden vermütlich zu den sogenannten Cysten." Entz (1907) has described, in the same species, a process which he interprets as conjugation, although he did not observe the "Zygospore" mentioned by Zederbauer. Jollos (1910), on the other hand, believes that, in *Ceratium tripos,* the process of encystment bears no relation to conjugation or other sexual process, although he does suggest autogamy as a remotely possible occurrence in encysted stages.

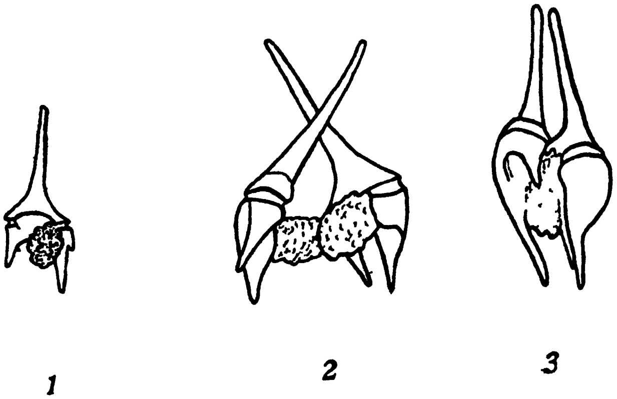

Fig. E. Series of stages in 'coupling' of *Ceratium hirundinella,* as figured by Entz (1924, figs. 6, 9, 8). (1) "Formation of the tubes of coupling with papillae"; (2) "coupled pair with long warty bridge" (3) "a coupled pair pressed closely to each other, with warty bridge" (Entz, 1924).

Encystment of *Ceratium hirundinella,* as observed in my material, seems to support the hypothesis that conjugation takes place in this species. The circumstantial evidence supporting this theory may be outlined as follows:

(1) Zederbauer (1904) has described "Kopulation" in living specimens of *Ceratium hirundinella,* and this process is said to be followed by the formation of a "Zygospore" from a mass of protoplasm joining the two conjugants. This zygospore is said to resemble very closely the ordinary cysts in size, structure, and general appearance. Entz (1907) later described, in the same species, a similar process which he interprets as conjugation. And in a more recent

paper, Entz (1924) has again described a coupling process in both living and preserved specimens, which are joined together by a mass of protoplasm (fig. E, 1–3), forming a sort of bridge between the two individuals. These *coupling* stages were shown by Entz (1924) to Daday, G. Entz, Sr., Filarszky, Klein, Mágocsy-Dietz, Apstein, Lohman, and Jörgensen, and all of these protozoologists were of the opinion that the coupling represented a case of sexual reproduction.

(2) Many of the cysts observed in my material contain two nuclei, which might be explained as contributions from the conjugants to a *zygospore,* or cyst, as described by Zederbauer and Entz.

(3) In numerous cases, one of the nuclei in the binucleate cysts is smaller than the other, and sometimes less than half the size (fig. D; pl. 9, figs. 39, 41, 43). It is interesting to note that such unequal nuclei are usually comparatively far apart in the cytoplasm; in most cases, although not in all, in which the two nuclei are close together, they are approximately equal in size. This inequality in size of the two nuclei is not so easily explained on the basis of a mitotic division of the primary cyst nucleus (unless by the possibility of reduction division); it suggests a comparison with the migratory and stationary micronuclei of conjugating *Paramecium.*

(4) Stages in which the nuclei of the cyst are markedly elongated or even constricted might just as readily be explained as the result of the fusion of gamete nuclei as by the preparation for a division of the nucleus.

(5) The chromosomes in the nucleus of the uninucleate cyst convey the impression that they are more numerous than those in the nucleus of the flagellate stage.

(6) Entz (1924) makes the following statements:

> In coupled Ceratia or in such ones which were detached of the coupling I often found . . . . two nuclei lying side by side and oriented, more or less alike. I suggested that these were nuclei wandered from one individual into the other. [Jollos conjectures autogamy!] I found amongst the coupled specimens also many cysts which were somewhat differing from the three-horned cysts known to me, in their more rounded outlines, coarse granular contents, and open apical horn. I found also cysts (and not exuviated forms, as Huber and Nipkow suppose, since I could verify the presence of a membrane!) which were joined together by the means of an empty cuirass (in two cases), as well as naked individuals connected by an empty cuirass, and naked specimens attached to individuals armoured with a cuirass (fig. 10). Since also all these pairs occupied a position against each other at 180° I suggested that this circumstance might have been explained by the supposition that in these cases, like in the formation of the zygospores of the Conjugatae, the plasma of one individual wandered into the other.

(7) Conjugation has also been described quite recently in another dinoflagellate, *Duboscquella* (Chatton, 1920).

(8) In addition, I have occasionally found in my stained preparations, flagellate stages in which the cytoplasm appeared normal, yet no nucleus was present. Such anucleate forms might have been left over from conjugation, supposing that the process occurs.

One of the most important discoveries within recent years in the cytology of the Protozoa was made by Dobell and Jameson (1915) in their work on the Sporozoa. These authors have shown that, in the forms studied (*Aggregata* and *Diplocystis*), fertilization involves a doubling of the number of chromosomes (resulting in the diploid number, and that this process is followed shortly by a reduction of the chromosomes to the haploid number—thus demonstrating beyond a doubt that sexual reproduction occurs in these forms. An interesting phase of this discovery is that the diploid number of chromosomes, established in the formation of the zygote, is soon lost as the result of an early reduction division; that is, the haploid number of chromosomes persists throughout almost the entire life-cycle of these Sporozoa.

Unfortunately, the number of chromosomes in *Ceratium* is so great that counting them, as was done by Jameson for the Sporozoa, is out of the question at present. But there is some evidence at hand, although inconclusive at best, to suggest the interesting possibility of a parallelism in chromosome behavior between *Ceratium hirundinella* and the Sporozoa described by Jameson. As mentioned above, both Entz and I have noted that the size of the chromomeres in the nucleus of the cyst is much smaller than that of the chromomeres in the nucleus of the flagellate stage. In addition, there seems to be no appreciable decrease in the amount of chromatin in the cyst, and the chromosomes themselves appear to be more numerous, if anything. Such facts might be assumed to indicate that the number of chromosomes in the cyst is possibly double that in the flagellate stage.

Furthermore, if it be assumed that conjugation of the flagellate stages leads to the formation of a 'zygote' cyst, as seems at least possible from the evidence at hand, the flagellate stage would contain, presumably, a 'gamete' nucleus with only the haploid number of chromosomes; while the nucleus of the 'zygote' cyst would contain the diploid number of chromosomes, and be comparable with the nucleus of the zygote in the Sporozoa. An hypothesis of this sort, although largely speculation, fits in with the descriptions of conjugation in *Ceratium* (Zederbauer, 1904; Entz, 1907, 1924).

In summarizing the situation in regard to the relation of encystment to sexual phenomena in *Ceratium,* it must be concluded that, at present, the evidence is insufficient to establish absolutely the occurence of sexual reproduction in this genus. The circumstantial evidence supporting conjugation is extensive enough, however, even at present, to preclude any denial that sexual reproduction may occur in *Ceratium hirundinella.*

SUMMARY

Ceratium hirundinella is shown to possess a typical neuromotor system, consisting of two flagella, their blepharoplasts, flagellar rhizoplasts, and an extranuclear centrosome. After their separation at the beginning of binary fission, the two daughter centrosomes remain connected for a time by a *paradesmose,* resembling the one described previously in *Oxyrrhis marina.*

Nuclear division, in contrast to the description of Entz (1921), is found to be a true mitosis, in which the chromosomes split longitudinally and then unfold to form an equatorial belt, or plate, composed of pairs of daughter chromosomes joined at their ends lying in the equatorial plane. There appears to be no true transverse division of the daughter chromosomes, such as that described by Entz.

The nucleus of encysted stages contains chromosomes similar to those found in vegetative individuals, and karyosomes are present at times; these chromosomes appear to be more numerous than those of flagellate stages, and their component chromomeres are distinctly smaller in size.

Additional evidence, supporting the hypothesis of sexual reproduction by conjugation in *Ceratium hirundinella,* is brought out in the discovery of binucleate cysts, possibly representing the products of conjugation in which each conjugant has contributed a nucleus to the cyst.

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EXPLANATION OF PLATES

PLATE 5

Ceratium hinundinella, flagellate stages. Unless otherwise stated, material is stained in iron-alum haematoxylin and figures are drawn at a magnification of × 1600.

Fig. 1. Nucleus in early prophase; note short scattered chromosomes. Bordeaux red followed by iron-alum haematoxylin.

Fig. 2. Surface view of nucleus in early prophase; note short chromosomes. × 960.

Fig. 3. Surface view of nucleus at a little later stage than figure 2; with longer chromosomes. × 960.

Fig. 4. Optical section of nucleus in later prophase; all chromosomes traced with camera lucida; each flagellar rhizoplast ends in a daughter centrosome on the nuclear membrane.

Fig. 5. Optical section of nucleus in prophase; flagellar rhizoplasts a little farther apart than in figure 4.

Fig. 6. Optical section of nucleus in prophase; all chromosomes traced with camera lucida.

Fig. 7. Optical section, at a deeper focal plane, of the nucleus shown in figure 6; all chromosomes traced with camera lucida.

Fig. 8. Optical section of nucleus of prophase.

Fig. 9. Optical section of nucleus of prophase, showing four karyosomes; note cytoplasmic inclusion near nucleus.

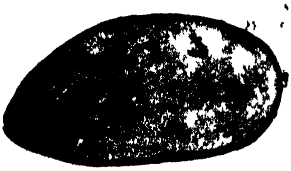
Fig. 10. Surface view of nucleus in prophase, showing single karyosome and chromosomes more or less radially polarized.

Fig. 11. Nucleus in prophase; note heavily stained fibril extending from the karyosome to a point outside the nuclear membrane.

Fig. 12. Nucleus in prophase; note fibril either on the upper surface of, or else inside the karyosome. Bordeaux red followed by iron-alum haematoxylin.

Fig. 13. Nucleus in prophase; similar to the "Knauel" stage figured by Schneider (1924).

Fig. 14. Note large cytoplasmic inclusion, apparently indenting the surface of the nucleus. Bordeaux red followed by iron-alum haematoxylin. × 960.



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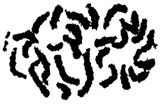

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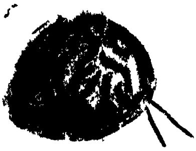

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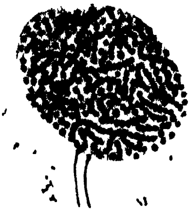

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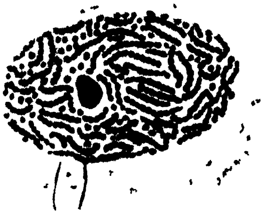

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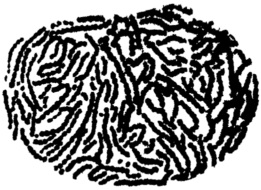

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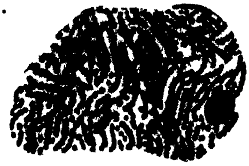

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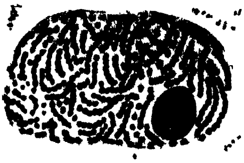

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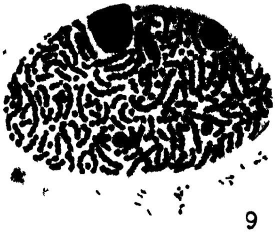

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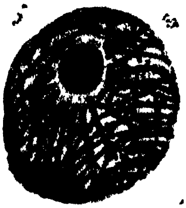

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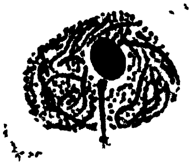

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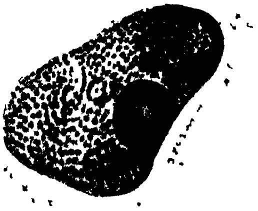

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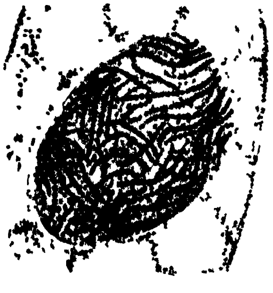

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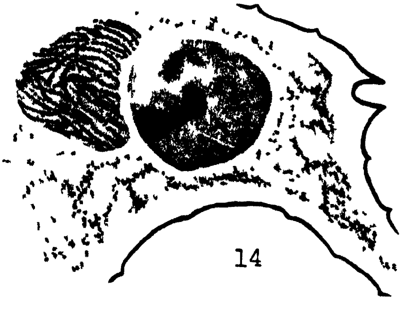

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PLATE 6

Ceratium hirundinella, vegetative stages. Unless otherwise stated, material stained in iron-alum haematoxylin and figures drawn at a magnification of × 1600.

Fig. 15. Nucleus in early metaphase, showing several chromosomes undergoing unipolar splitting. Note paradesmose joining the two daughter centrosomes, to each of which are connected two flagellar rhizoplasts. 7.5μ section.

Fig. 16. Surface view of the end of a nucleus in the early metaphase; several chromosomes in various stages of unipolar splitting.

Fig. 17. Nucleus in early metaphase, with several splitting chromosomes. 7.5μ section.

Fig. 18. Surface view of nucleus in early metaphase, showing several loops of double chromosomes formed by splitting.

Fig. 19. Nucleus in early metaphase, showing a number of unfolding pairs of daughter chromosomes. 7.5μ section.

Fig. 20. Nucleus in early metaphase. Bordeaux red followed by iron-alum haematoxylin.

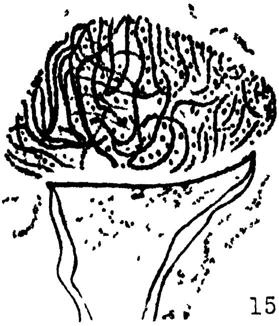
Fig. 21. Early metaphase; unfolding of daughter chromosomes well under way. 7.5μ section.

Fig. 22. Early metaphase; the daughter chromosome pairs are beginning to straighten out. 7.5μ section.

Figs. 23, 24. Metaphase nuclei. 7.5μ sections.

Fig. 25. Later metaphase; chromosomes almost completely straightened out in the equatorial plate.

Fig. 26. Metaphase plate; chromosomes beginning to separate in equatorial plane. × 1200.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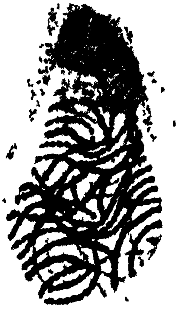
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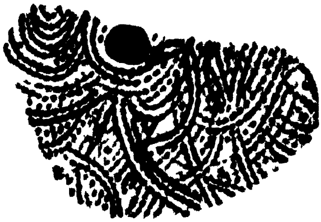
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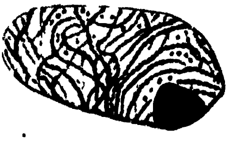
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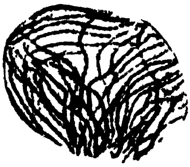
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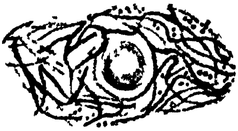
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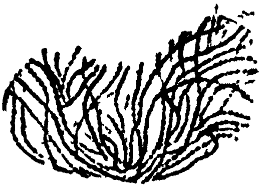
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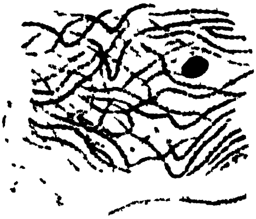
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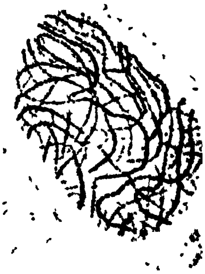
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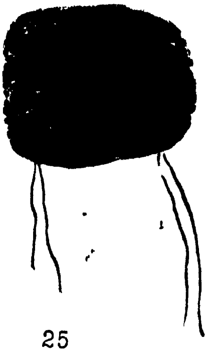
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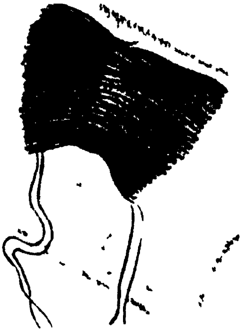
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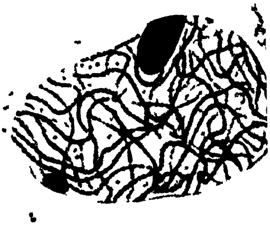
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PLATE 7

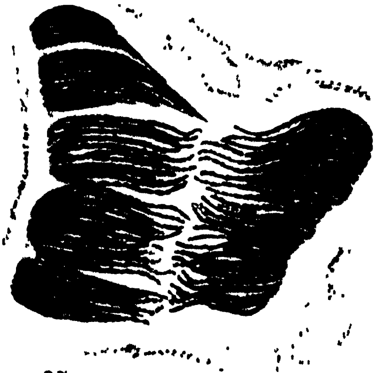
Ceratium hirundinella, vegetative stages. Unless otherwise stated, material stained in iron-alum haematoxylin and figures drawn at a magnification of × 1600.

Fig. 27. End of metaphase, with daughter chromosomes completely separated in the equatorial plane.

Fig. 28. Anaphase-telophase; chromosomes in one of the nuclei still retaining their parallel arrangement. × 960.

Fig. 29. Anaphase-telophase; nuclei shown in surface view; the earlier parallel arrangement of the chromosomes is beginning to disappear. × 1200.

Fig. 30. Anaphase-telophase; chromosomes beginning to show typical coiled arrangement as found in the prophase. Bordeaux red followed by iron-alum haematoxylin.



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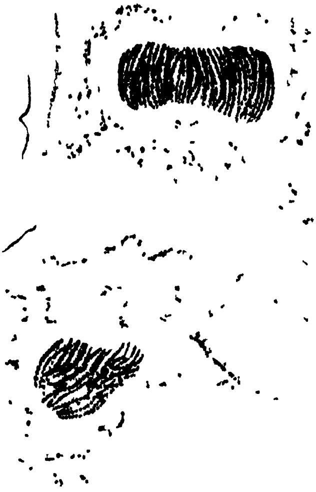

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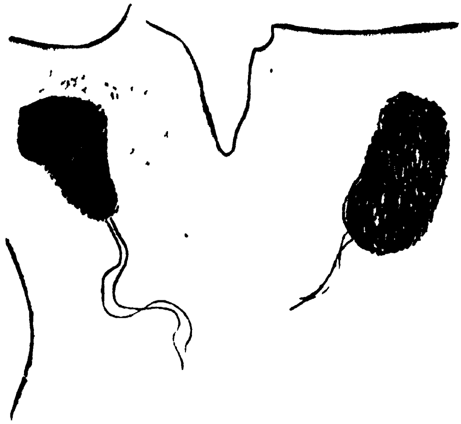

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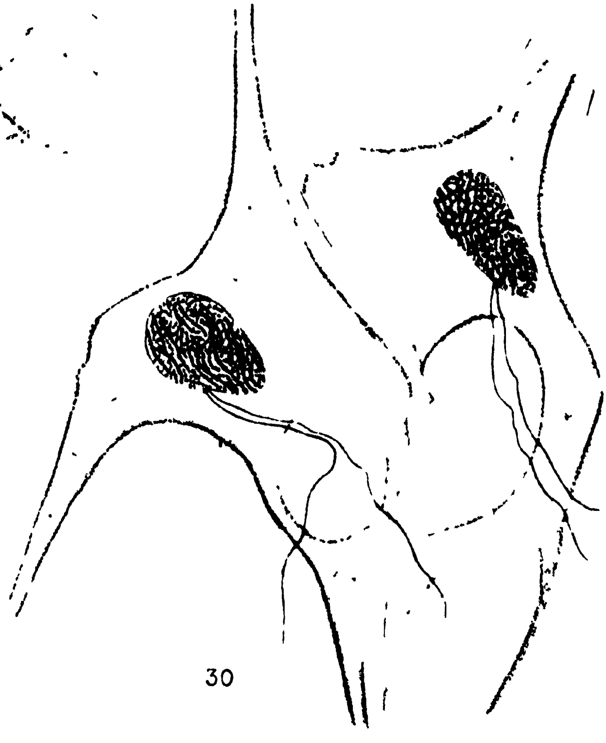

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PLATE 8

Ceratium hirundinella, encysted stages. Material stained in iron-alum haematoxylin and figures drawn at a magnification of × 1600, unless otherwise stated.

Fig. 31. Optical section of nucleus of cyst, showing distinct chromosomes. 7.5μ section.

Fig. 32. Nucleus of cyst containing coiled chromosomes; portion of cyst wall shown. 7.5μ section.

Fig. 33. Optical section of nucleus containing a single karyosome, and distinct chromosomes. 7.5μ section.

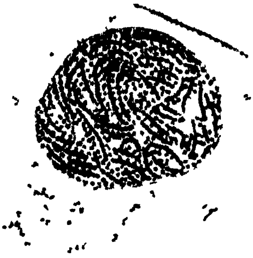
Fig. 34. Optical section through elongated nucleus of cyst. 7.5μ section.

Fig. 35. Surface view of elongated and U-shaped nucleus of cyst. 7.5μ section.

Fig. 36. Surface view of elongated nucleus, U-shaped, smaller end at a deeper focal plane than the larger. 7.5μ section.

Fig. 37. Elongated nucleus of cyst, surface view; note amphiaster-like body in cytoplasm. 7.5μ section.

Fig. 38. Surface view of elongated nucleus of cyst, with ampiaster-like body in cytoplasm. 7.5μ section.



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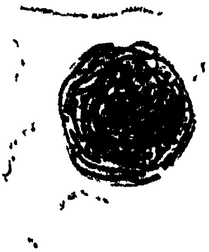

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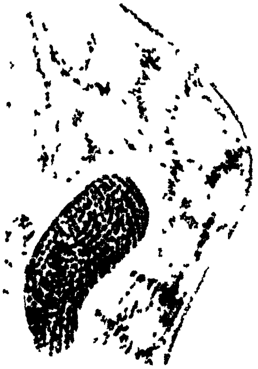

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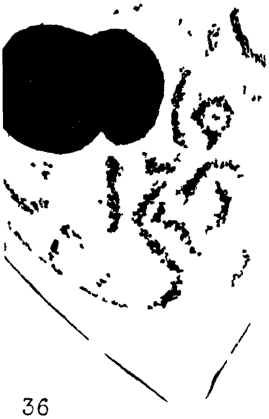

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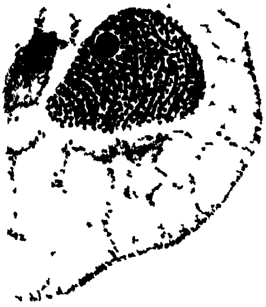

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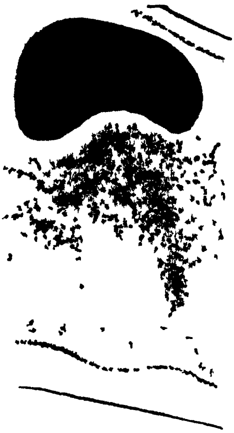

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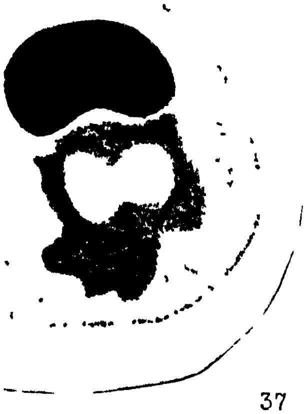

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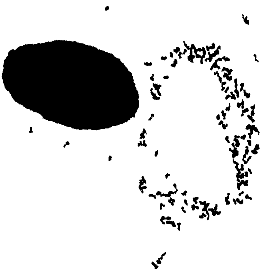

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PLATE 9

Ceratium hirundinella, encysted stages. Material stained in iron-alum haematoxylin and figures drawn at a magnification of × 1600, unless otherwise stated.

Fig. 39. Binucleate cyst; optical sections through nuclei; nucleus on right is elongated and constricted. 7.5μ section.

Fig. 40. Two nuclei apparently connected by cytoplasmic fibrils. 7.5μ section.

Fig. 41. One nucleus is elongated and the other is approximately spherical, as determined by focusing up and down. Bordeaux red followed by iron-alum haematoxylin.

Fig. 42. Binucleate cyst; unequal nuclei connected by cytoplasmic fibrils. 7.5μ section.

Fig. 43. Two unequal nuclei connected by cytoplasmic fibrils. 7.5μ section.

Fig. 44. Binucleate cyst; nuclei close together and almost equal in size. × 960.

Fig. 45. Binucleate cyst; two nuclei almost equal in size, close together, and imbedded in a dense mass of cytoplasm, fibrils of which appear tc extend between the two nuclei. Bordeaux red followed by iron-alum haematoxylin.

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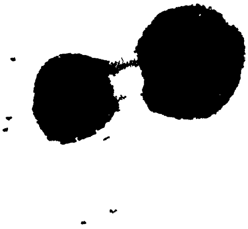

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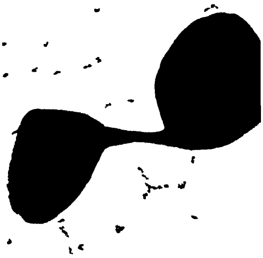

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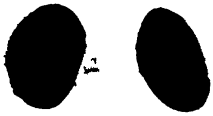

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THE CULTIVATION OF ENDAMOEBA GINGIVALIS (GROS)

BY

BEATRICE FAY HOWITT

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CONTENTS

PAGE
- I. Introduction 66
- II. General methods 68
  - 1. Culture medium 68
    - (1) Solid egg slants 68
    - (2) Locke's solution 69
    - (3) Locke-albumen solution 69
  - 2. Inoculation 70
    - (1) Fresh material 70
    - (2) Material from cultures 70
  - 3. Routine examination and count of amoebae 70
  - 4. Test of the hydrogen ion concentration 71
  - 5 Methods for making permanent stained slides 71
- III. Cultivation of *Endamoeba gingivalis* (Gros) 73
- IV. Morphology and behavior 75
  - 1 Material from the mouth 75
  - 2. Cultures 77
    - (1) Description of living forms 77
    - (2) Description of *E. gingivalis* from stained slides 78
    - (3) Behavior of *E gingivalis* 79
    - (4) Division stages of *E. gingivalis* in fresh preparations 81
    - (5) Bacteria 84
- V. Experimental work with cultures of *E gingivalis* 85
  - 1. Relationship of the hydrogen ion concentration to the amoeba cultures 86
    - (1) Curves showing the daily relationship of the pH to the number of amoebae over a 5- or 6-day period 86
    - (2) Determination of the pH range for the growth of *E. gingivalis* 89
    - (3) Growth curve of *E gingivalis* and its relationship to the pH 93
  - 2 Omission of different salts from the Locke's solution 96
  - 3 Addition of varying percentages of 10 per cent glucose to a sugar free medium 101
  - 4. Experiments with lipoids 102
  - 5. Addition of proteins and amino acids to the different media 107
    - (1) Locke-Lewis medium 107
    - (2) Peptone 108
    - (3) Cystin 108
    - (4) Tyrosin 110
  - 6. Addition of blood to cultures of *E gingivalis* 111
    - (1) Strain No 38 with addition of human blood 111
    - (2) Strain No. 38 with addition of rabbit blood 114
    - (3) Strain No. 13 with addition of both human and rabbit blood 116
- VI. Effect of low temperatures upon the growth of *E. gingivalis* 116
- VII. Summary 118
- VIII. Literature cited 120
- IX. Explanation of plates 122

I. INTRODUCTION

For many years protozoologists have endeavored to cultivate the entozoic amoebae of animals and of man but without any great success. Positive reports, especially of the cultivation of *Endamoeba dysenteria* (*histolytica*), have appeared only to be later discredited and the cultures proved to be those of some free-living form. Within the past year, however, Boeck and Drbohlav (1924) have reported the successful maintenance of this *Endamoeba* in an artificial medium that is reliable and suitable for its needs. They obtained a strain of *E. dysenteriae* from an active dysenteric case and have maintained it under cultivation for many months. Kofoid and Wagener (1925) have recently confirmed this work of Boeck and Drbohlav, as they have been able to maintain several strains of *E. dysenteriae* for four or five months.

The success obtained by these workers with *E. dysenteriae* suggested the feasibility of applying the same methods to the cultivation of *Endamoeba gingivalis,* the ámoeba found in the mouth. This organism apparently has not as yet been successfully maintained under artificial cultivation, although attempts have undoubtedly been made. Howe (1916) endeavored to cultivate *E. gingivalis,* using Ross's jelly, horse-serum, and saliva, respectively, but with negative results. Wherry and Oliver (1916) were able to maintain an amoeba from the mouth for several days in a modification of Martin's (1911) "pleuritic agar" medium. This report, however, is very inconclusive, since no figures are given and no mention is made of transplantation or further continuance of the strain. Drew and Griffin (1917) describe the occurrence of two amoebae from the mouth; a large one corresponding to the description for *E. gingivalis,* and a smaller one of the free-living *Vahlkampfia* type, which they designate as *Amoeba buccalis.* They believe the latter to be a free-living form that has become semiparasitic and adapted to life in the pus pockets of pyorrhoea cases, since it is often found, along with *E. gingivalis,* in such an environment. They were unable to cultivate the latter amoeba but give a complete description of its life-cycle and of the behavior of the other form under artificial cultivation.

Amoebae from the mouth have been variously described by different authors, but the consensus of opinion now is that the amoebae are all identical and that the names given (*Amiba buccalis* Steinberg [1862], *Amoeba dentalis* Grassi [1879], *Amoeba kartulisi* Doflein [1901], *Amoeba oralis hominis* Le Wald [1907], *Endamoeba pyogenes* Verdun and Bruyant [1907], and *E. confusa* Craig [1916]) are synonyms for the form originally described by Gros in 1849 as *Amoeba gingivalis.* This organism was found to have the type of nucleus of the genus *Endamoeba;* the generic name was therefore changed and the nomenclature of *Endamoeba gingivalis* (Gros) is now used.

Prior to 1914 this organism was considered merely a harmless commensal of the human mouth, but following the work of Bass and Johns (1914), of Smith and Barrett (1915), and of Evans and Middleton (1915), much controversy has been waged in regard to the relationship of this amoeba to the disease known as pyorrhoea alveolaris. The authors mentioned firmly believed that *Endamoeba gingivalis* was the specific cause of the disease, because of its frequent association with this condition and the apparent healing of the processes under emetin treatment. This belief, however, was later discredited because of the failure of the drug to cause a permanent cure. Interest in the organism then waned, but, as the question in respect to pathogenicity has never been satisfactorily answered, the whole matter is still in abeyance. It would seem that the solution in respect to its positive or negative relationship to pyorrhoea alveolaris will be obtained only when the amoebae are grown in an artificial medium, isolated in pure culture, and inoculated into animals or man with the outcome either of the production or the non-production of the disease. The present research was undertaken to lay a foundation for further work along this line by determining whether or not *E. gingivalis* would grow under artificial conditions and, should growth be maintained, what would be its behavior in mixed bacterial cultures.

The work was made possible through grants to the California Stomatological Research Group from the Carnegie Corporation, from the American Dental Association, and from the Associated Radiograph Laboratories of San Francisco. The author is also indebted to Professor Charles A. Kofoid for his interest and his helpful suggestions, to Mr. Anthony Salle for preparation of the standard buffer solutions, and to Mr. Horace Child for his kindness in bringing cultures from cases in the State Prison at San Quentin.

II. GENERAL METHODS

1. Culture Medium

The medium used in both the experimental work and the routine transplantation of the organisms was essentially that described by Boeck and Drbohlav (1924). As a solid base they used coagulated egg slants containing a small amount of Locke's solution. To these slants a liquid medium was added, consisting either of a mixture of eight parts of Locke's solution to one part of the human serum, or of Locke's solution containing 1 per cent crystallized egg albumen, both media being sterilized by filtering through a Berkefeld candle. The Locke's solution they used, however, contained 2.5 gms. glucose to a liter, or 0.25 per cent, which was ten times the quantity usually employed in this fluid. Through what later proved to be a fortunate accident, this amount of glucose was omitted from the Locke's solution used in the present work and the formula was made up with 0.25 gms. (0.025 per cent) glucose to the liter instead of the 2.5 gms. (0.25 per cent). As successful cultivation of *E. gingivalis* occurred with the lower percentage of glucose, and as the organisms entirely died out with the higher, the former was continued as the standard amount. While *E. gingivalis* grew well in a liquid medium of Locke's solution and human serum in the proportions given, yet it was found that equally as good results were obtained in a fluid containing the whites of two eggs to a liter of Locke's solution. This medium was also used by Boeck and Drbohlav. Since it was more difficult to obtain the serum, this egg albumen medium was used in the routine for many months.

The methods for making the media were as follows:

(1) *Solid egg slants.*—Eight fresh eggs were washed free from dirt, wiped, and left in 90 per cent alcohol for 15–30 minutes to sterilize the shells. The eggs were then carefully cracked into a liter flask containing glass beads, 100 c. c. of Locke's solution were added, and the whole mixture was shaken until homogeneous. It was then tubed through a sterile tubing apparatus and sterilized in the autoclave for 20 minutes at 15 lbs. pressure. By closing all valves and not allowing any steam to escape during this period, the slants were evenly coagulated without any air bubbles and were ready for immediate use. Boeck and Drbohlav first used the inspissator to

coagulate the egg and then sterilized later in the autoclave. The first mentioned method, however, proved to be quicker and fully as efficient.

(2) *Locke's solution.*—The formula for the Locke's solution used was:

NaCl	9 gms.
$CaCl_2$	0.2 gms.
KCl	0.42 gms.
$NaHCO_3$	0.2 gms.
Glucose	0.251 gms.
Distilled H_2O	1000 c.c.

The Boeck-Drbohlav formula contained 2.5 gms. glucose and only 0.4 gms. KCl.

This solution was sterilized in the autoclave at 15 lbs. pressure for 15 minutes. The utmost care had to be used in both the making and the sterilization of this solution, as the amoebae were extremely sensitive to any variation in the salt content. The liquid should normally be water clear. If there is the slightest tinge of brown or if there is a white precipitate (probably of $CaCo_3$), the fluid should be discarded. Prolonged sterilization often causes these changes to take place.

(3) *Locke-albumen solution.*—Two eggs were cleaned and sterilized in 90 per cent alcohol, and the whites were very carefully cracked into a sterile liter flask containing glass beads, great care being taken to avoid contamination. First, the whites were shaken with the beads and then one liter of sterile Locke's solution was added. The mixture was well shaken until the egg seemed dissolved. It was found necessary to adjust the reaction since the medium had a tendency to gain in alkalinity upon standing. Eighteen to twenty c. c. of sterile N/20 HCl, added with a pipette, proved to be about the right amount to add to a liter of fluid in order to give pH 7.4–7.6, a very favorable reaction for growth.

This egg albumen medium was usually not filtered, as it was found that the amoebae grew well in the small flakes of undissolved albumen which often precipitate out, and which were removed by the filter. By taking great care in the preparation of this medium and by running an uninoculated control tube at the time of every transplant, filtration did not seem to be necessary. Recently, however, filtered Locke-egg albumen, containing only one egg white to a liter of Locke's fluid has been used with very good results. This Locke-egg-albumen medium is added aseptically in 6–10 c. c. amounts, either to sterile tubes or to the egg slant medium.

2. INOCULATION

(1) *Fresh material.*—Most of the work was done on material from two easily available sources and the inoculations were made directly into the desired medium from scrapings taken with a sterile, sharpened applicator stick around the gingival margins of the teeth or else from the depths of a pus pocket. The medium was previously warmed to 37° C. and then placed immediately in the incubator after inoculation. Fresh cover-glass preparations were usually made at the same time to determine the presence of the amoebae. Permanent stained slides were also made.

(2) *Material from cultures.*—Cultural transplants were made by removing a small amount of material with a sterile, capillary pipette from the deposit in the bottom of the culture tube and inoculating this substance into the fresh medium, warmed to 37° C. Great care was always taken not to allow any culture tube to become chilled by too long a removal from the incubator. Transplants were usually made every other day into the Locke-egg slant-albumen (L.E.A.) medium and incubated at 37° C.

3. ROUTINE EXAMINATION AND COUNT OF AMOEBAE

A standard method of examination was used so that the recorded results would be comparable, although not truly quantitative. Since the tubes usually contained from 8 to 10 c. c. of fluid medium, 1 c. c. of this liquid could easily be removed from the top with a pipette without disturbing the sediment containing the amoebae. This amount was placed in a small (4 × ½ inch) test tube, calibrated to the 1 c. c. mark, and the tube was set aside to be used in the determination of the hydrogen-ion concentration.

A small amount of material was removed from the bottom of the culture tube and placed on a warm slide. A 22 × 22 mm., No. 1 cover glass was applied and any superfluous fluid was removed from the edges with the pipette, so that a fairly uniform preparation was obtained. The slide was then placed in a warm chamber (37° C.) on the microscope stage and examined with the 16 mm. objective and × 6.4 oculars on a compound binocular microscope. The amoebae could be easily seen under this magnification of 240 diameters, the eye being quickly attracted by their motility and by their peculiar refractive waxy appearance.

Beginning at the upper left-hand corner of the cover glass, all of the amoebae were counted in the area of 130 fields. Since the 16 mm. objective came below the rim of the warm stage, the number of fields was restricted on two sides, thus providing a convenient counting chamber; the other two sides were limited by the edges of the cover glass. Although the depth was not regulated, yet, as the amoebae always settled to the substrate within a few minutes and as the counts were always performed with the same technique, the results were comparable though not quantitative. As a rule the counts seldom ran very high, from 5 to 100 or 150, so that the enumeration was not difficult. If they went above 200 or 300, however, undoubtedly many organisms were passed over. When the amoebae were in agglomerated clumps, the counts were also inaccurate. Considering the number of inaccuracies, it is surprising how uniform the results often were.

4. Test of the Hydrogen-ion Concentration

The hydrogen-ion concentration was determined by the colorimetric method, on the 1 c. c. of cultural material placed in the small test tube, 0.1 c. c. of the desired indicator being added to this fluid. The tube was shaken, placed in a wooden-block comparator, and the color compared in transmitted light through a white background, with the color of the standard buffer solution in a tube of the same caliber, according to the method described by Hurwitz, Meyer, and Ostenberg (1915).

5. Methods for Making Permanent Stained Slides

Permanent slides were made by fixing the wet preparation in hot Schaudinn's solution and staining by the usual iron-haematoxylin method. Precautions were taken to lower the slides into the fixing fluid in a horizontal rather than a vertical position in order to prevent the washing off of the amoebae.

Slides were also stained by Gram's method, after fixation in methyl alcohol. Wright's stain was used occasionally, applied on the direct preparation while the latter was slightly moist.

TABLE 1

SUMMARY OF THE RESULTS FROM THE DIFFERENT CULTURES

Culture No.	Pyorrhoea alveolaris			Pyorrhoea condition unknown	Other conditions	Smears from mouth showing amoebae	Amoebae found only in		Positive transplants made	Cultures showing Trichomonads	Duration of cultivation
	Medium	Slight	None				1st tube	Transplants			
13		+				+		+	68 to date		10/21/24 to 4/8/25
22		+				+		+	27		11/11/24 to 1/7/25
32				+	Abscessed tooth extracted	No smears	+		−	+	
33	+					+		+	2	+	1/29/25 to 2/1/25
34	+				Syphilis	+	−	+	2	−	1/20/25 to 2/1/25
35	+					+		+	6	−	2/3/25 to 2/12/25
36	+					+		+	22	−	2/3/25 to 3/15/25
37	+					+	−	−	−	+	
38	+					+		+	18 to date	−	2/26/25 to date (4/8/25)
39		+				−		+	1	+	3/14/25 to 3/17/25
40			+			+	Only 1 +	−	−	−	3/14/25 to 3/15/25
41					Pyorrhoea treated with bacterine material from cavity	+		−	−	+	
42				+		No smears		+	13 to date		3/14/25 to date (4/8/25)

[1] Date, April 8, 1925.
+ = Present
− = Negative.

III. CULTIVATION OF ENDAMOEBA GINGIVALIS (GROS)

Most of the work on the culture of *E. gingivalis* was carried on with strains from two sources: one strain (cultures No. 13 and No. 22) from a case with a fairly normal mouth, showing slight gingivitis, and the other strain (culture No. 38) from a case having a deep pyorrhoeal pocket which contained numerous active amoebae. In order to show that *E. gingivalis* can be cultured from other mouth sources as well as from those given, material was obtained from eleven men in the State Prison at San Quentin, in whose mouths pyorrhoea was present in varying stages, and in some, absent altogether. These cases were all diagnosed by Dr. G. W. Simonton according to the routine methods employed by the dentists of the California Stomatological Research Group.

The first attempts at culture were made on September 3, 1924, inoculations being made into the Locke-serum-egg slant medium of Boeck and Drbohlav. Amoebae were found in the 24-hour cultures and upon later transplantation, but the organisms soon died out. A number of unsuccessful trials were made and much experimentation performed in respect to the kind of medium to use and its proper adjustment, before real success was attained. The successful culture, No. 13, planted in the Locke-serum-egg slant medium on October 21, 1924, was later subcultured into the Locke-egg albumen-egg slant medium (L.E.A.) and is still growing (April 8, 1925), after 68 transplants. The latter were made about every second day into the L.E.A. medium, although the organisms often remained viable in the cultures for a much longer period. Nearly all of the experimental work was performed with organisms from strain No. 13.

Another culture (No. 22) was started from the same person on November 11, 1924. It was continued until January 7, 1925, when, after 27 transplants, it was accidentally lost.

Culture No. 38 was planted on February 26, 1925, from the mouth having the bad pyorrhoeal pocket, and has been continued with vigorous growth until the time of writing (April 8, 1925), after 18 subcultures into L.E.A. medium.

Table 1 shows the results from the cultures obtained at San Quentin, together with a general summary of all the strains. Culture No. 32 was of a whole tooth which had been dropped into the culture medium from a person who had an abscess of long duration. Six amoebae were found after 24 hours' incubation, together with hundreds of trichomonad flagellates, *Tetratrichomonas buccalis* Goodey (Goodey and Wellings, 1917). No amoebae were found on transplantation and the cultures soon became putrefactive and overgrown with the flagellates.

Culture No. 33 from a case of moderate pyorrhoea showed 25 amoebae after 24 hours. Two successful transplants were made showing many amoebae and also a few flagellates. Culture No. 34 was from a man who had moderate pyorrhoea. No amoebae were found in the original tube, but a very few were noticed in 2 transplants, after which the organisms disappeared.

Culture No. 35, from a case of moderate pyorrhoea with deep pockets, was successfully carried from February 3 until February 12, 1925, with 6 transplants. It was then discontinued. Culture No. 36, also from a moderate case of pyorrhoea, showed many amoebae in 48 hours. It was carried from February 3 to March 15, 1925, with 22 transplants. Culture No. 37 from a similar case of moderate pyorrhoea showed no amoebae in either the original tube or in the transplants; trichomonads, however, were present. Culture No. 39 from a slight pyorrhoea case showed no amoebae in the original tube but several in the first transplant. Many flagellates were present and the amoebae soon died out. As *E. gingivalis* was not found in the direct smear from the mouth it could not have been present in very large numbers. Culture No. 40 from a person who had no pyorrhoea showed a few amoebae on the direct smear from the mouth and one organism in the culture. No further growth occurred, so they could not have been very numerous. Culture No. 41 from a patient under treatment with the bacterine for a long-standing pyorrhoea showed several amoebae on the direct smear from the mouth but none in the original culture or subcultures. Culture No. 42 was obtained from material removed from a cavity. The pyorrhoea condition of the patient was unknown, but a very heavy growth of *E. gingivalis* was obtained upon subculture. This strain, planted on March 14, 1925, is being continued with 12 transplants to date (April 8, 1925), and is proving to be a very active and vigorous culture.

Summary of results—

1. Of the cultures from 12 different individuals, 10 were positive for *E. gingivalis,* although 2 of these had the organisms only in the

original tube. Flagellates were present in one of these tubes, so that the amoebae were probably overgrown, while in the other tube only one amoeba was found.

2. Direct smears were obtained from only 9 of these cases. Eight of these showed *E. gingivalis,* while the ninth, although it did not show the organism on the smears, developed it upon cultivation. Only once, therefore, was the amoeba found in the cultures when not in the smears, and only once was the smear positive and the culture negative. It is likely that material obtained again from these cases would be positive. Of the ten patients whose condition in regard to pyorrhoea was known, all except one showed some form of the disease, and of these all except one gave positive cultures. Amoebae were found in both the smears and the cultures from the one patient without pyorrhoea. Whenever trichomonads were present the amoebae were few in number and soon died off.

3. Although cultures were made from so few cases, yet there is ample evidence to show that *E. gingivalis* grows readily in mixed bacterial cultures in the media described above, and there is every reason to believe that, with suitable care, such cultures can be carried on indefinitely.

IV. MORPHOLOGY AND BEHAVIOR

1. Material from the Mouth

Endamoeba gingivalis has been variously described by different authors, reference being made especially to the following: Bass and Johns, 1915; Smith and Barrett, 1915; Craig, 1916; Mendel, 1916 *a, b*; Goodey and Wellings, 1916; Goodrich and Mosely, 1916; Nowlin, 1917 *a, b*; Fischer, 1917. The most recent and the most adequate morphological account of the organism, however, is that of Kofoid and Swezy (1924). Through a very careful nuclear study they have been able to clearly differentiate this organism from *Endamoeba dysenteriae,* the amoeba of the bowel, with which it might occasionally be confused on account of certain superficial resemblances. By thus establishing an undoubted distinction between the two forms, the failure of emetin treatment in cases of pyorrhoea alveolaris may be explained, since this drug is specific for *E. dysenteriae* and apparently has no permanent effect upon *E. gingivalis.*

The following description for *E. gingivalis,* as found in the mouth is typical for the organisms obtained from No. 13 and No. 38, the only cases available for fresh preparations.

Endamoeba gingivalis is a large active amoeba with a slight yellowish-green tinge, showing a sharp distinction between the ectoplasm and the endoplasm, and usually with a single broad hyaline pseudopod. The number and shape of the pseudopodia may be variable, but they always remain relatively free from inclusions in contrast to the much congested condition of the endoplasm. The latter is frequently quite filled with very large food vacuoles containing ingesta of various shapes and sizes (pl. 10, fig. 1), presumably nuclei of leucocytes or remains of salivary corpuscles (Goodey and Wellings, 1917). Ingested bacteria may also be found, and occasionally a red blood cell. These large inclusions give the organism a characteristic appearance in the fresh preparation as well as in the slides stained with iron haematoxylin.

The nucleus is not often noticed in the fresh smear on account of the number of inclusions, but when visible it is seen to be spherical, large, and vesicular, with refractile beads of chromatin on the inner membrane. Occasionally a central refractile granule (karyosome) may be noticed.

The amoebae from all the cases described above, as found on the stained slides, conformed essentially to the description of *E. gingivalis* given by Kofoid and Swezy (1924). The large, deeply staining inclusions were always present and the nuclei were comparable to those described and figured by these authors.

Figure 2, plate 10, showing an amoeba stained from a preparation from the mouth of No. 38, indicates the typical open form of karyosome made up of several deeply staining irregularly arranged granules surrounded by a more lightly staining, finely granular zone, through which faint, spoke radii extend to the beads or plaques of chromatin on the inner side of the nuclear membrane. A fairly clear zone is to be noticed between the granular halo and the nuclear chromatin. Figures 3 and 4, plate 10, show the same type of nucleus from stained material obtained from No. 13 and No. 40.

2. CULTURES

(1) *Description of living forms.*—All the strains of amoebae cultured from the different cases appeared to be morphologically similar both in the fresh preparations and when stained by iron-haematoxylin.

In 24- or 48-hour subcultures in L.E.A. medium the amoebae are ordinarily quite active, often with the same greenish-yellow tinge noticed in the organisms from the mouth, and with the same waxy appearance of the pseudopodia. The latter are variable in size and shape, according to the age of the organism, the type of culture medium, and the reaction. When observed in L.E.A. medium (pH 5.2–5.8) just after division, the amoebae are spread out on the substrate with irregular or scalloped ectoplasmic processes and a much vacuolated endoplasm (fig B). At a later stage, the pseudopodia become more elongated and blade-like, always clear; although in old or unfavorable cultures, the hyaline portion is small and the granules flow rapidly out to the tip. They may protrude from any part of the animal and in several directions at once (pl. 10, fig. 3). Quite often the animal appears to be going two ways at the same time. One side, however, soon gains ascendency and the remainder of the body is slowly dragged in the dominant direction. A very much elongated form is thus produced.

The amoeba seems to secrete an adhesive substance largely from the posterior region, or the end opposite the direction of locomotion, since forms are often seen dragging along large clumps of bacteria intermixed with débris (pl. 11, fig. 12). Bacteria may also be noticed adhering to other parts of the thick pellicle, and when red blood corpuscles have been added to the slide preparation, as many as twenty have been found attached to the organism as it crawls along. Kofoid and Swezy (1924) describe the same adhesive quality for organisms directly from the mouth.

The nucleus is usually very clearly visible in the living cultural forms, especially in acid media (pH 5.0–5.8). It is large, spherical, or slightly ovoidal, and the ring of chromatin beads on the nuclear membrane stand out distinctly. The central karyosome may or may not be visible. The whole nucleus seems flexible and as if capable of compression like a rubber ball. It is usually noticed in the anterior region of the endoplasm, close to the line between endoplasm and ectoplasm. In a medium of a higher pH (pH 6.2–7.0) the nucleus is not so distinct.

The motile amoebae often show pinkish vacuoles of different sizes, some of them containing bacteria. Numerous granules may also be present, especially in older cultures. These granules may be in the form of small cocci, or as a grayish, amorphous substance, undoubtedly obtained from the egg slant. Refractile lipoidal-like bodies varying in size, and with a slightly irregular outline, may often be present. These bodies are to be found both free in the slide preparation and in small pieces of yolk removed from the medium.

(2) *Description of E. gingivalis from stained slides.*—When the organism is stained by iron haematoxylin the nucleus is the most prominent structure (pl. 10, fig. 2) because of the absence of the large chromatin-staining bodies that appear in preparations from the mouth. On stained slides from egg slant cultures, however, the animal may show round bodies of various sizes, enclosed in clear areas, and staining a very faint gray color (pl. 12, figs. 15, 16). These bodies will be further discussed later. Bacteria may also be noticed in the amoeba, and sometimes long chains of *Streptococci* (pl. 12, fig. 8).

While there is not the very sharp differentiation between endoplasm and ectoplasm in the stained amoebae such as may be noticed in the living forms, yet one can usually recognize some distinction between them. The endoplasm stains a darker gray, is more granular, has different-sized vacuoles, and contains the nucleus and the food inclusions, while the ectoplasm is a lighter gray and has a slightly different texture.

The stained nucleus is large (average 3.2μ), the dimensions depending upon the size of the individual organism, and contains a central karyosome composed of several (3–4) deeply staining chromatic grains, often arranged as a square, a ring, or a cross, and surrounded by a halo of finely granular material. A clearer zone, through which the spoke radii extend, is found between these granulations and the chromatin material on the nuclear membrane. The latter is distinct and bears deeply staining chromatin material, arranged either as regular beads on the inner surface or as more irregular plaques, massed in different locations on the membrane.

Slides studied from all the strains cultured show amoebae conforming to this general type (compare figures on pls. 10 and 11), although as one might expect there were a few individual variations. This description also conforms to that for the amoebae from the mouth material (pl. 10, fig. 1), and to that given by Kofoid and Swezy

(1924) for *E. gingivalis.* It seems reasonable to conclude, therefore, that the organisms obtained in mixed bacterial culture were the same as those in the original material from which the cultures came and that they all conform to the description for *E. gingivalis* (Gros).

(3) *Behavior of E. gingivalis.*—Since the amoebae are unable to swim about in a fluid medium in the manner of the flagellates, it is necessary to supply them with a substratum upon which to creep. For this reason all media containing the solid egg slant substance are the most favorable for prolonged cultivation. It is necessary, however, to have a liquid above the slant as the organisms soon die from lack of moisture and are unable to subsist on the egg alone.

The amoebae are found on the surface of the slant; in the bacterial sediment with clumps of débris; and in any small, broken off flakes of coagulated egg. They seem to have a tendency to crawl into something and hide, although apparently they do not penetrate very far into the egg. Paraffin sections of an egg culture showed no amoebae below the surface, but, as it was difficult to make successful sections of the cooked egg, more work should be done in this connection before drawing any definite conclusion.

On slide preparations of the motile forms, hundreds of amoebae were often found crawling on thin pieces of egg or buried within these pieces in agglomerated masses. Often an amoeba would crawl out to the edge of the mass and, extending a pseudopod toward the surrounding fluid, feel about in several directions in the manner of an earthworm under stimulation. Probably finding an unfavorable environment, it would withdraw into the mass again.

In an acid L.E.A. medium the amoebae are active in young cultures. They may exhibit all manner of strange movements; remaining in one place on the substrate and reaching up toward the cover glass with a pseudopod, in the fashion of a measuring worm, and then doubling back again to the flat surface; flowing first in one direction and then doubling back to the opposite side; or one may attach itself to the slide by the adhesive posterior end, lengthen itself out, feel around in a semicircle, and then suddenly pull loose the sticky end, bringing that end of the body forward like the snapping of a rubber band. In this medium there is also a tendency for the organism to be somewhat spread out with broad, fan-like pseudopodia (pl. 11, fig. 11). Elongated, finger-like, or banana-shaped forms, however, are also numerous (pl. 11, fig. 12).

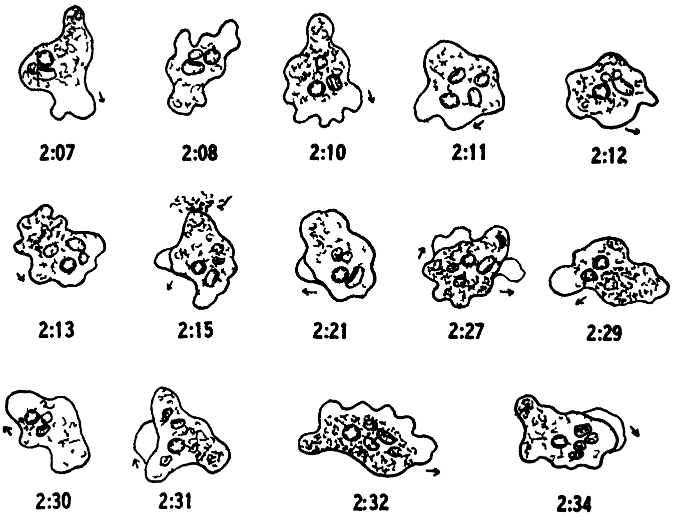

Fig. A. The pseudopodial formation of an amoeba from a culture of strain No. 38, covering a period from 2:07 P.M. to 2:34 P.M. Several ingested red blood cells are shown. Camera lucida drawings × 600.

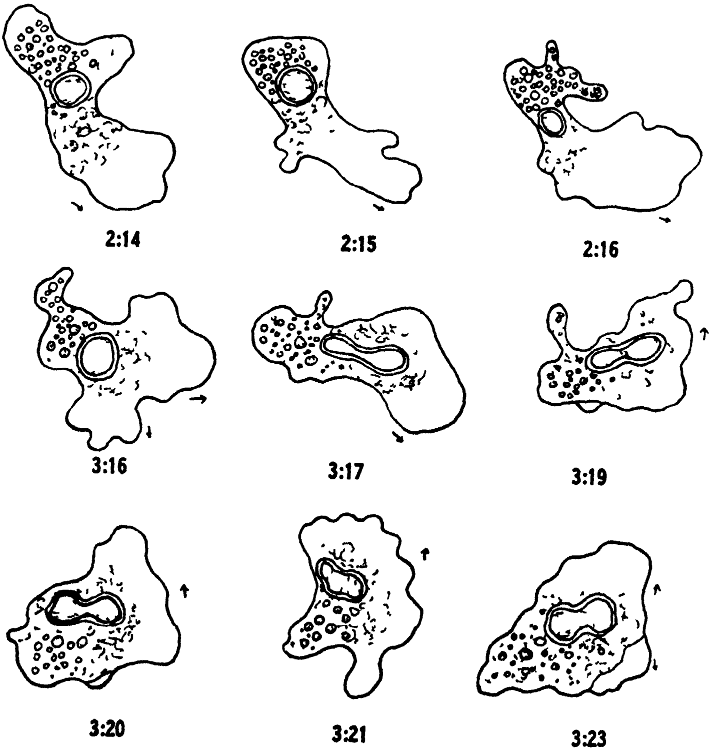

Fig. B. The pseudopodial formation of an amoeba from a culture of strain No. 13. The numbers show the time intervals from 2:14 P.M. to 3:23 P.M. One large lipoidal yolk body and many smaller ones are shown in the organism. No nucleus is shown. Freehand sketches.

Figures A and B may give an idea of the movements of this organism in fresh preparations from L.E.A. cultures. Figure A was drawn under the camera lucida and shows the action of No. 38 for a period of about sixteen minutes. Figure B is a freehand sketch of No. 13 and shows the presence of one large yolk body and several smaller inclusions.

Although the solid egg substratum is most favorable for artificial cultivation, yet *E. gingivalis* can also be grown in a fluid medium of the Locke solution and egg white alone. The final pH in this medium was usually between pH 6.2 and pH 7.0. The unfiltered fluid is more suitable than the filtered, because the small flakes of albumen which settle out seem ncessary for the maintenance of the amoebae. If favorable conditions are present, the organisms in 18–20 hours may be found in large numbers in masses of débris and bacteria entangled in albumen flakes, or else they may be extended in an orderly fashion on long albuminous threads. When in these masses or on the threads, they are either motionless or very sluggish, exhibiting only a slight squirming movement. The protoplasm seems thickened, and the amoebae, instead of being spread out and vacuolated, appear more rounded or ovoid. One might easily mistake them for cysts, until careful observation detects the slight motion. Although cysts have been reported for *E. gingivalis* by some authors (Chiavaro, 1917, Smith and Barrett, 1915, Craig, 1916, Nowlin, 1917), yet no true cysts, such as are formed by *E. dysenteriae,* have been noticed in preparations from any of these strains. Forms very much resembling cysts have been observed but more cytological evidence must be obtained before making a definite statement.

(4) *Division stages of E. gingivalis in fresh preparations.*—While so far no division forms have been found on the stained slides, yet, as only a small number of those on hand have been examined, it is hoped that a report may be made later in respect to the number of chromosomes and the mitotic structure. Binary fission, however, was witnessed on eight different occasions in fresh preparations from several cultures (No. 3, No. 13, No. 33, No. 38). The performance was similar in all cases except one (fig. D). The length of time before complete separation of the daughter cells occurred varied slightly in each instance but that was probably due to the stage of mitosis reached before the organism came under observation. Figure C is a freehand sketch of division stages of an amoeba from a 48-hour culture of No. 13 in L.E. medium containing 1 per cent cystin.

The amoeba was first observed at 5:35 P.M. flattened out on the subtrate and very much elongated with a constricted portion in the center. The ectoplasm was fan-like and there was no very marked difference between it and the endoplasm. The latter was vacuolated, devoid of all inclusions except one small, shiny yolk body which remained in one daughter cell. No definite nuclear structure could be noticed. At 5:37 P.M. the two extended lobes pulled farther apart,

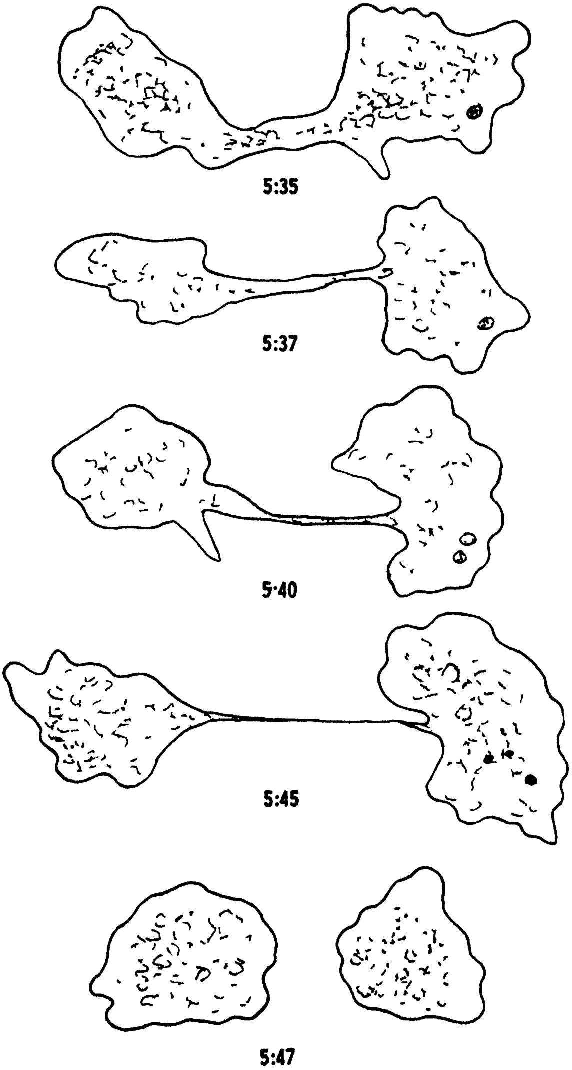

Fig. C. Successive division stages of one amoeba from a 48-hour culture of strain No. 13. Observed time interval was from 5:35 P.M. to 5:47 P.M. Freehand sketches.

leaving a very narrow ectoplasmic strand stretched between them. This gradually become thinner and thinner, as the two daughter cells moved farther apart, but it persisted for about 10 minutes. At 5:48 P.M. the connection suddenly snapped and the two new amoebae remained some distance from each other in the same flattened out condition with irregular ectoplasmic borders. After several minutes they began to push out broad pseudopodia and before long they crawled away like the usual forms.

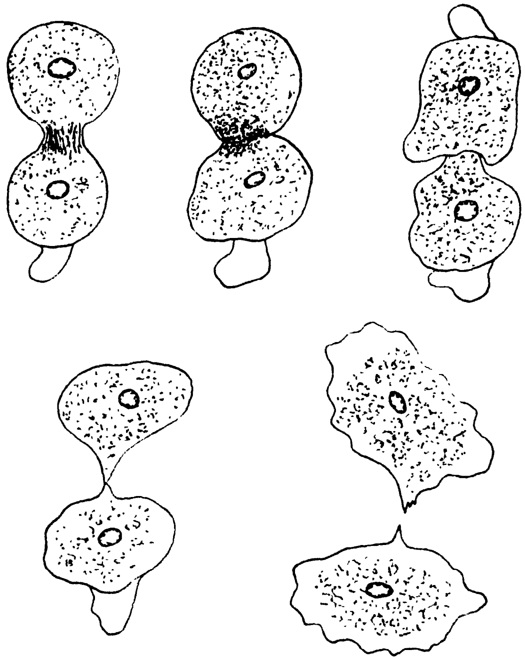

Fig. D. Succesive stages in the division of an amoeba from a 10-hour culture of strain No. 13. Freehand sketches

With one exception, division occurred in the same manner for the other forms, although the connecting strand did not remain so long between the cells as in this instance. The actual division of the nucleus was never observed. Figure D indicates a slightly different occurrence that was noticed in a 10-hour L.E.A. culture of No. 38.

At 7:45 P.M. two somewhat rounded amoebae with thickened protoplasm, were noticed in the position of the first figure. The pellicle of the two forms seemed to have fused and formed a connection between them, inside of which there was distinct protoplasmic streaming. A nucleus was observed in each amoeba. The two forms gradually approached each other so that the connection was no longer visible. A convulsive movement then seemed to agitate both amoebae and they suddenly pulled apart, leaving ragged strands of protoplasm at the place of former union. This unusual behavior might easily be interpreted as a form of conjugation, which has been described for *E. gingivalis* by Craig (1916). The performance was most suggestive of conjugation, yet it is not certan whether there was witnessed a coming together of two individuals or the last stages of division after the nucleus had divided and passed into the the daughter cells. Only a stained slide, showing the condition of the chromosomes, would settle the point.

(5) *Bacteria.*—Several futile attempts were made to obtain *Endamoeba gingivalis* free from bacteria, but it was finally decided to leave this task for a later date. No true estimate of the activities of the organism can be obtained, however, until it is grown in pure culture, so that it is a matter of prime importance to make an effort toward purification.

In the present cultures no particular trouble was taken to identify all the bacteria, but the usual gram positive type of mouth flora, *Staphylococci* and *Streptococci*, were the dominant forms. *Leptothrix*-like rods and spiral organisms were occasionally noticed in the preparations from freshly isolated cultures, but they seemed to disappear after prolonged cultivation. Blood agar plates inoculated from No. 13 soon after the culture was started showed almost a pure culture of the alpha type of *Streptococcus.* Plates inoculated again from the same strain after five months of transplantation, showed the same *Streptococcus.* Anaerobic cultures were not made, so that one cannot be certain what other organisms were also carried along. Culture No. 38 contained mainly *Staphylococcus albus* and a hemolytic *Streptococcus.*

Amoebae containing bacteria were often found on stained slides from both these strains. The bacteria were usually cocci, diplococci, or even long chains of streptococci (pl. 11, fig. 8). When stained by Gram's method, gram positive cocci and diplococci could often be seen in the endoplasm.

V. EXPERIMENTAL WORK WITH CULTURES OF E. GINGIVALIS

Although the cultures of *E. gingivalis* were not bacteria-free, some experimental work was attempted in order to gain an approximate idea of the range of hydrogen-ion concentration within which growth occurs; of the rate of growth and the relationship of the latter to the pH of the medium; and the effect of certain carbohydrates, lipoids, amino acids, and proteins upon the growth of the organism. Its behavior toward the addition of red blood corpuscles was also to be considered. Since the bacteria in the cultures were essentially those associated with the same amoebae in the patient's mouth, it might happen that these experiments would give some clue to the relationship of *E. gingivalis* to the normal environment. A later correlation of this data with the work of the biochemists in regard to diet factors and salt metabolism in pyorrhoea alveolaris, might perhaps give some idea as to the rôle played in the disease by this amoeba.

For the sake of clarity, the following experimental work is arranged in the form of tables, which record the pH of the culture medium and the number of amoebae counted on the same date of observation. A negative mark means that no organisms were found and the following abbreviations were used: L.E. = Locke-egg slant; L.A. = Locke-albumen; L.E.A. = Locke-egg-slant-albumen; L. = Locke's without egg slant or albumen. The methods of counting and of obtaining the pH have already been described. Observations were usually made every 24 hours for 3 or 4 days unless otherwise stated.

In all this experimental work, one must bear in mind the number of variable factors which may be present. A broad interpretation must be placed upon the results, and the presence or absence of the amoebae must be noted rather than the exact numbers. The inaccuracies in regard to the counts have already been explained. The other main variable was not being able to estimate the exact number of amoebae planted in each tube. The same number of drops of cultural sediment were usually placed from a pipette into each tube, but there was no guaranty that amoebae would be equally distributed in all the drops. This may account in some cases for the scarcity of amoebae in the first 24 hours and their subsequent increase in the following 48 or 72 hours. The few organisms originally inoculated needed time to increase before they could be noticed.

1. RELATIONSHIP OF THE HYDROGEN-ION CONCENTRATION TO THE AMOEBAE CULTURES

(1) *Curves showing the daily relationship of the pH to the number of amoebae over a 5- or 6-day period.*—Charts A, B, and C indicate the type of growth curve observed for cultures of *E. gingivalis* and the daily relationship between the number of amoebae and the pH of the medium. The curves in charts A and B represent daily obser-

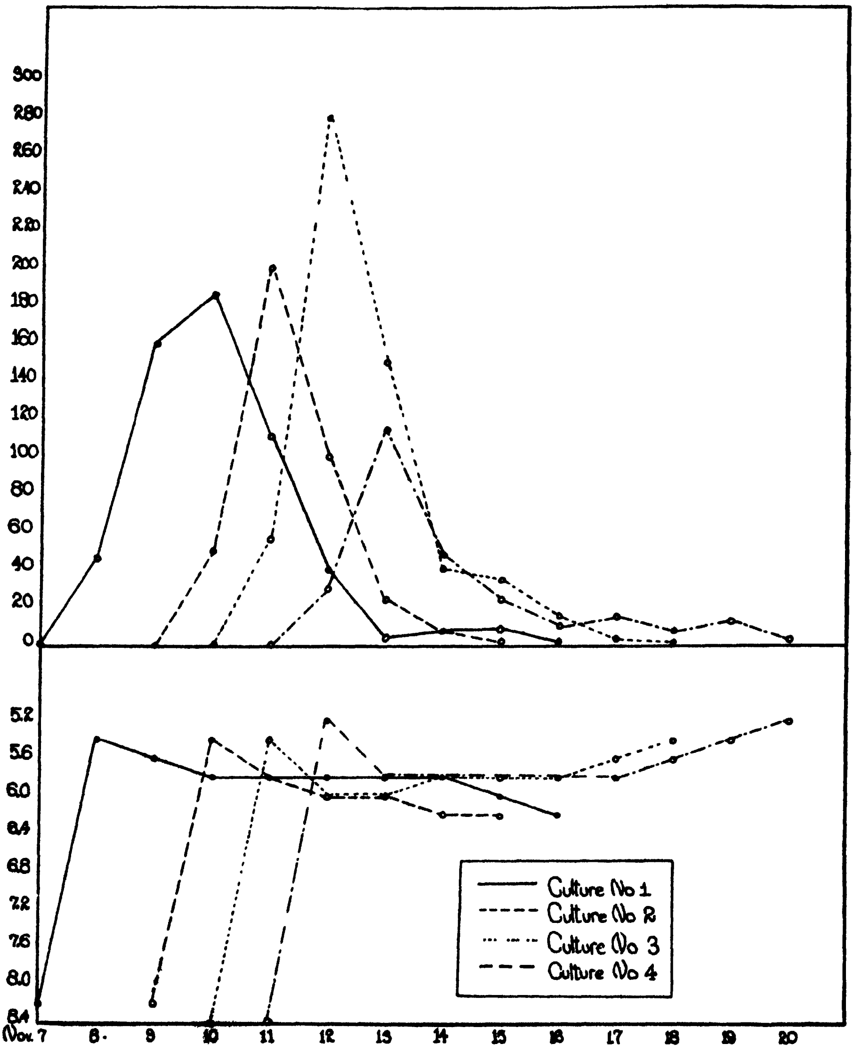

Chart A

vations made on the two strains, No. 13 and No. 22, over a 5- or 6-day period. The results are shown from 4 culture tubes of L.E.A. medium planted on different days from No. 22 and of 3 tubes of L.E.A. inoculated with No. 13. The number of amoebae and the pH of the medium were recorded every 24 hours, until the culture fluid was either exhausted or no organisms were found. Chart C shows the curves from 4 cultures of No. 13 in L.E.A. medium, to which fresh

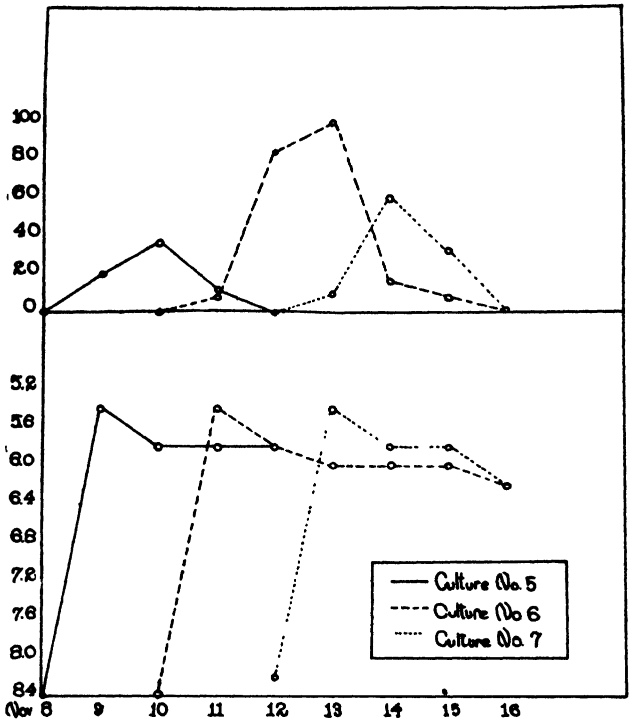

Chart B

Locke-albumen solution was added on the days marked on the chart with an asterisk. The curves with the upper base line represent, in all three charts, the number of amoebae counted from the different tubes, plotted against the days on the base line. The lower curves represent the observed pH of each medium plotted also against the days. It is therefore possible to read from the two sets of curves, using No. 1, chart A, for example, what are the number of amoebae (188) and the corresponding pH (pH 5.8) for the same day (Nov. 10). A few dates overlapped, so that lines from different cultures frequently ran along together.

The main points of interest in this chart are to be observed in the peaks of the curves. While the actual number of organisms enumerated is of no very great significance, because of the different variables,

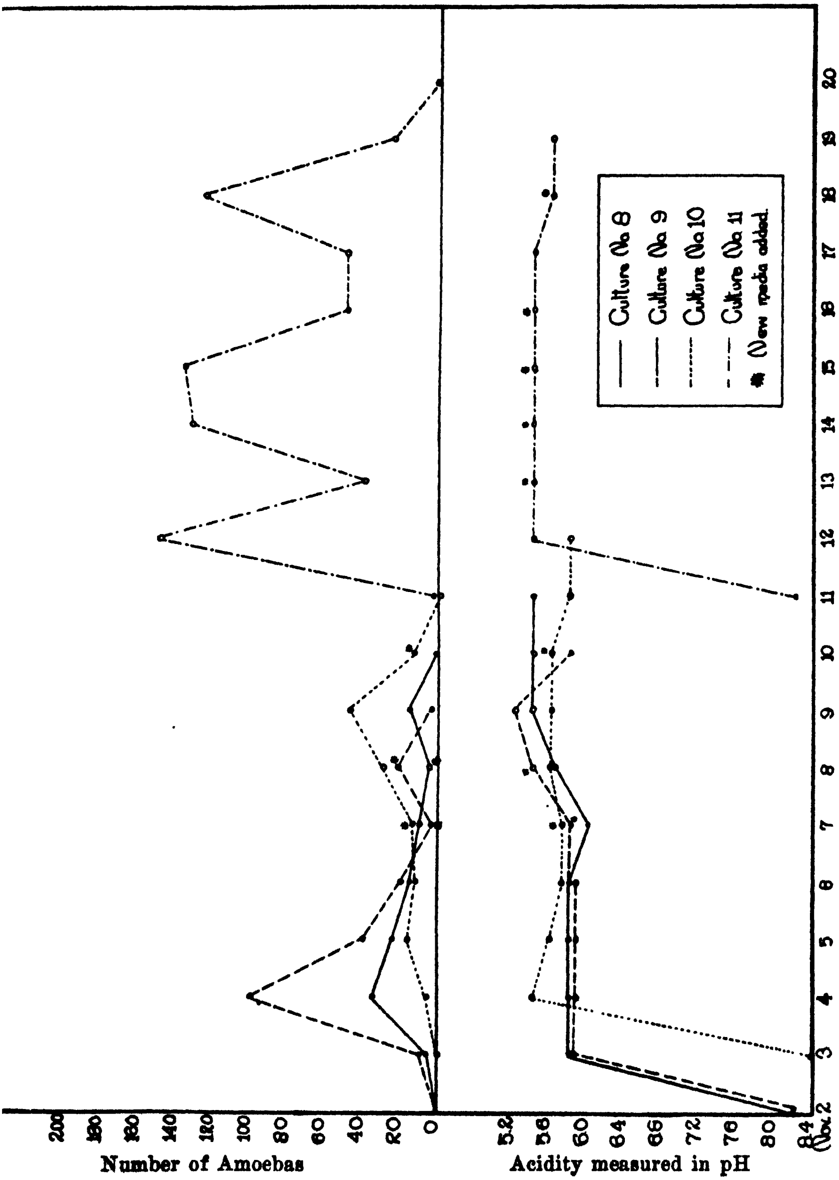

Chart C

it is of interest to note that the amoebae increase to a certain maximum on a particular day and then rapidly die off on the successive days. Forty-eight hours seems to be the time of maximum growth in the majority of curves (tubes 2, 3, 4, 5, 7). That the time was prolonged to 72 hours in tubes 1 and 6 might have been due to the fewer numbers of organisms originally inoculated. The curves in chart C show that, when the amoebae begin to die off, probably from the effects of accumulated waste products, chiefly bacterial, they may be stimulated to an increase in growth by the addition of fresh medium. The second high peak, however, seldom reached the height of the first, except in tube 10.

The lower curves are typical for such carbohydrate-fermenting bacteria as the *Streptococci* and *Staphylococci* in the cultures. The hydrogen-ion concentration increases sharply to a maximum, when the carbohydrates are being fermented, and then there is a slight decrease in acidity and the maintenance of a more or less level curve without a drop to the base line. In comparing the peaks of the upper and the lower curves, it is found that the maximum number of amoebae occur when the pH is about 5.8. This is apparently a favorable acidity for growth and for the maintenance of the organism, since the medium often remains at this constant for several days. The rises in acidity toward the end of the observations in tubes 3 and 4 were probably caused by the scarcity of fluid in the tube and the accumulation of bacteria in the portion withdrawn for pH determination.

It may be observed from these charts, therefore, that *E. gingivalis* has the usual biological type of growth curve; a rise to a maximum and a subsequent decline; that the amoebae may be maintained in a tube of L.E.A. medium for a considerable length of time (9 days for tube 11) provided fresh solution is added; and that pH 5.8 is a favorable hydrogen-ion concentration for multiplication.

(2) *Determination of the pH range for the growth of E. gingivalis.*—The following experiments were performed to determine the effect of inoculating cultures of *E. gingivalis* into media with a wide range of initial pH. On November 9, 1925, 12 tubes of L.E.A. medium with a pH range from 5.0 to pH 9.0 made by adding small amounts of either N/20 HCl or N/20 NaOH to the original L.A. solution, were each inoculated with 4 drops of sediment from several cultures of No. 13. Observations in regard to the number of amoebae and the degree of pH were made on each tube for 3 successive days, the results being shown in table 2.

It is evident that, no matter what was the initial pH, in 24 hours the bacteria tended to bring all the tubes to nearly the same level. With the exception of the first tube, all those below pH 7.0 became pH 5.0, and all those above pH 7.0 varied from pH 5.2 to pH 5.6 in an ascending order. On the following days the pH increased to a more or less constant average of pH 5.6. Increasing numbers of amoebae were found on each day from all the tubes, except the first one, which dropped to pH 4.4. in 24 hours, and the one with the initial pH of 8.6. The latter discrepancy may have been due to the inoculation of only a small number of amoebae, since the organisms were able to withstand the higher pH of 9.0. Although on the third day amoebae were obtained in large numbers from the tubes of lower pH, yet the range between pH 7.0 and pH 7.8 seems more favorable, since the amoebae were present in the first 24 hours, and increased rapidly on the following days.

TABLE 2

THE EFFECT OF PLANTING CULTURE NO. 13 IN L.E.* MEDIUM ADJUSTED TO VARYING HYDROGEN-ION CONCENTRATIONS

Date of planting: January 24, 1925

Initial pH	24 hours		48 hours		72 hours	
	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count
3.0	4.2	......	4.4	43	4.9	42
3.4	4.6	10	5.5	12	5.6	55
5.0	5.4	27	6.4	50	6.4	15
5.4	5.6	18	6.4	25	6.6	12
5.6	5.8	29	6.6	12	6.6	17
6.2	5.8	18	6.4	3	6.6	1
6.4	6.0	8	6.4	32	6.6	10
6.8	6.2	4	6.6	18	6.6	9
7.4	6.0	44	6.6	98	6.6	13
7.8	6.0	8	6.5	112	6.6	12
8.2	6.2	37	6.6	45	6.6	7
9.0	6.2	15	6.6	29	6.6	5

* L. E. = Locke-egg slant.

The experiment was repeated with strain No. 13, on February 24 (table 3), using the Locke-egg slant medium without the albumen. Tubes of lower initial pH (pH 3.0 and 3.4) were also included. Cultures in this medium did not remain at so low a general level of acidity as in the L.E.A. medium but tended to average around pH 6.4–6.6 after the first 24 hours. Amoebae were found in all of the tubes, showing their ability to withstand a wide range of initial pH. *In most cases the number increased to a maximum on the second day* and the highest counts were made in the tubes between *pH 7.4 and* pH 7.8.

TABLE 3

THE EFFECT OF PLANTING CULTURE NO. 13 IN L.E.A.* MEDIUM ADJUSTED TO VARYING HYDROGEN-ION CONCENTRATIONS

Date of planting: January 9, 1925

Initial pH	24 hours		48 hours		72 hours	
	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count
5.0	4.4	....	5.0	.....	5.2	.....
5.4	5.0	....	5.3	35	5.4	190
5.8	5.0	1	5.3	13	5.4	45
6.2	5.0	....	5.6	19	5.6	190
6.6	5.0	..	5.6	27	5.6	140
7.0	5 2	27	5.6	57	5.6	410
7.2	5.2	1	5 6	9	5.6	30
7.6	5.2	16	5.6	70	5.6	102
7.8	5.2	7	5 6	36	5.6	85
8.2	5.2	4	5.6	3	5.8	.....
8 6	5.4	.....	5 3	1	5.8	1
9.0	5.6	4	6.0	30	6.0	34

* L. E. A.=Locke-egg slant-albumen.

Although the foregoing results are not conclusive, it is evident that *E. gingivalis* is able to survive the shock of being placed in fluids of a wide range of hydrogen-ion concentration (pH 3.0–9.0) and that, as soon as the bacteria have brought the medium to the normal level of pH, the amoebae multiply and continue to live as usual.

Table 4 shows the effect of correcting the pH to a constant every 24 hours for 5 days, by adding either sterile N/20 HCl or N/20 NaOH. Tubes each with a different pH value were used.

Culture No. 22 was inoculated into 4 tubes of L.E.A. medium with a pH range of 5.6, 6.8, 8.2, and 8.6, respectively. A few organisms were maintained in the first culture but as the tube was accidentally broken no further observations could be made. Growth was obtained in all of the cultures, the highest counts being in the tube of pH 8.2.

The apparent result of the daily correction of the pH in cultures in L.E.A. medium is to maintain the number of organisms at about the same level. The growth curves do not show the sharp peaks seen in chart A, but rise to a certain point and are then maintained along a level line.

Since the bacteria increased the hydrogen-ion concentration so quickly and tended to bring the reaction of all the tubes to an average *pH, the experiment was tried of correcting the reaction at different* intervals for 24 hours, in order to discover whether the continued action of a definite pH had any effect on the growth of the amoebae. At 11 P.M. March 13, 1925, 10 tubes of L.E. medium in duplicate, with a varying range of pH from pH 3.0 to pH 8.4, were each inoculated with an equal number of drops of material from 4 or 5 different

TABLE 4

THE EFFECT OF CORRECTING THE HYDROGEN-ION CONCENTRATION TO A CONSTANT OVER A PERIOD OF 5 DAYS

Culture No. 22 in L.E.A. medium

Initial pH	Planted		Date	Examined		Corrected pH	Amoeba count
	Date	Time		Time	pH		
5.6	11/10/24	5:00 P.M.	11/11/24	4:15 P.M.	5.0	to 5.6	......
			12/12/24	2:50 P.M.	6.0	to 5.6	5
			11/13/24	4:00 P.M.	6.0		18—tube broken
6.8	11/10/24	5:00 P.M.	11/11/24	4:30 P.M.	6.8		2
			11/12/24	3:05 P.M.	6.2	to 6.8	1
			11/13/24	4:15 P.M.	6.4	to 6.8	34
			11/14/24	4:35 P.M.	6.4	to 6.8	27
			11/15/24	3:10 P.M.	6.2		33—material gone
8.2	11/10/24	5:00 P.M.	11/11/24	4:50 P.M.	5.8	to 8.2	2
			11/12/24	3:15 P.M.	6.4	to 8.2	9
			11/13/24	4:35 P.M.	6.8	to 8.2	74
			11/14/24	4:54 P.M.	6.8	to 8.2	50
			11/15/24	3:20 P.M.	6.8		40—material gone
8.6	11/10/24	5:00 P.M.	11/11/24	5:00 P.M.	5.4	to 8.8	1
			11/12/24	3:30 P.M.	6.4	to 8.8	6
			11/13/24	5:00 P.M.	6.8	to 8.6	50
			11/14/24	5:55 P.M.	7.2	to 8.8	50
			11/15/24	3:40 P.M.	7.0		30—material gone

cultures of No. 13. The pH of each culture was then determined at 8:30 A.M. the following morning, nine and a half hours later. In all, except the first 2 tubes, the pH had dropped to between pH 5.0 and pH 5.4, as had occurred in the previous experiments. The amoebae were not counted. The reaction of one set of tubes was then adjusted to the original pH values. The reaction of the other set was left unchanged.

The same procedure was repeated every 4 hours throughout the day until 8:30 P.M. when an examination was also made for amoebae. The tubes were examined again on the following day to see if any amoebae had been present in those cultures which were negative on the previous occasion. The tubes in the control set were left unchanged during the whole period.

In all the experimental tubes, a few amoebae were able to survive this drastic treatment, but apparently no multiplication occurred except in the one with pH 5.4. Although some amoebae were found in all of the control tubes, the optimum growth occurred between pH 7.0 and pH 7.8. On the whole it appeared that the control tubes gave better results with respect to growth than the others, showing that the hardy individuals which survived the first shock of the initial pH preferred the conditions normally brought about by the bacteria.

As a result of these experiments it appears, therefore, that, in general, *E. gingivalis* is able to survive inoculation into media with a wide range of pH, but that the optimum growth occurs when the initial hydrogen-ion concentration lies between pH 7.0 and pH 7.8.

(3) *Growth curve of E. gingivalis and its relationship to the pH.*—Many futile attempts were made to determine the growth curve of *E. gingivalis* when inoculated directly into media from the mouth. More convincing results were obtained, however, when cultures of No. 13 and of No. 38 were used. The amoebae were planted into a series of tubes containing equal amounts of the same medium, observations being made at definite intervals for 14 hours. In order to complete the series for 24 hours, tubes of the same medium were inoculated the evening before and observed 14 or 15 hours later, with hourly or 2-hourly observations thereafter until the period was completed. Although the results were not very accurate on account of the uncertainty in regard to the number of organisms inoculated, yet some general idea was obtained in respect to their activities.

Tables 5 and 6 compare the results of the hourly or 2-hourly observations on cultures of No. 13 and of No. 38 in L.A. and L.E.A. media, respectively. The experiments with No. 13 in both these media were performed on separate dates and in a different lot of Locke-albumen solution from those with No. 38. The results, however, are fairly comparable, although the bacteria in No. 38 produced a lower pH than those in No. 13.

Part I of both tables shows the amoebic growth when the two strains are cultured in L.A. medium. The organisms tend to round up when first inoculated into the fresh medium and apparently many degenerate and disappear. (Only the motile forms or the viable rounded ones were counted.) During the first 6 hours the amoebae seem to be adjusting themselves to the new environment. The less vigorous ones probably die and the hardy forms survive. This period seems to correspond to the so-called period of "lag" in bacterial growth, since, as soon as the bacteria began to multiply and the pH went down, the amoeba counts started to rise. The rise began between the eighth and tenth hours in both cases.

TABLE 5

EXPERIMENT WITH THE 2-HOURLY EXAMINATION OF CULTURE No. 13 OVER A 24-HOUR PERIOD

	Tube No.	Number of hours after planting	Initial pH	Observations	
				pH	Amoeba count
I. L. A. Medium	1	2	7.8	7.2	12 motile, 14 rounded up.
	2	4	7.8	7.0	10 motile.
	3	6	7.8	7.0	25 motile, many in clumps.
	4	8	7.8	7.0	35 motile, some in clumps.
	5	10	7.8	6.6	45 motile, many in clumps.
	6	12	7.8	6.0	130 motile, uncountable number in clumps.
	7	14	7.6	5.6	30 motile.
	8	16	7.6	6.0	1000+, mostly in clumps.
	9	18	7.6	7.0	1000+, uncountable.
	10	20	7.6	7.0	70 free, 1000+ in clumps.
	11	22	7.6	7.0	300 free, 1000+ in clumps.
	12	24	7.6	7.0	170 free, 1000 in clumps.
II. L. E. A. Medium	1	2	7.8	7.0	29 motile, 5 rounded up.
	2	4	7.8	5.8	5 motile.
	3	6	7.8	5.8	6 motile.
	4	8	7.8	5.8	————
	5	10	7.8	5.6	6 motile, many in clumps.
	6	12	7.8	5.6	————
	7	14	7.8	5.6	40, many in clumps.
	8	16	7.6	5.4	27 motile.
	9	18	7.6	5.6	6 motile.
	10	20	7.6	5.6	385 motile.
	11	22	7.6	5.6	100 motile, many in clumps.
	12	24	7.6	5.6	200 motile, uncountable number in clumps.

Between the thirteenth and fourteenth hours, the lowest acidity was attained for both No. 13 and No. 38. The amoebae, however, did not reach their maximum numbers in either case until the pH had risen slightly. Unfortunately the organisms in the No. 13 cultures

TABLE 6

EXPERIMENT WITH THE 2-HOURLY EXAMINATION OF CULTURE NO. 38 OVER A PERIOD OF 20 TO 24 HOURS

	Tube No.	Number of hours after planting	Initial pH	Observations	
				pH	Amoeba count
I. L. A. Medium	1	2	7.6	7.0	6
	2	4	7.6	6.8	17
	3	6	7.6	6.8	19
	4	8	7.6	6.4	86
	5	10	7.6	6.0	45
	6	12	7.6	5.8	88
	7	13	7.6	5.6	150
	8	14	7.6	6.8	130
	9	15	7.6	6.8	240
	10	16	7.6	6.8	4—Many dead amoebae.
	11	17	7.6	6.8	Many dead amoebae.
	12	18	7.6	6.8	Many dead amoebae.
	13	19	7.6	7.0	Many dead amoebae.
	14	20	7.6	7.0	Many dead amoebae.
II. L. E. A. Medium	1	2	8.0	6.0	57 (division stages noted).
	2	4	8.0	5.2	24
	3	6	8.0	5.2	7
	4	8	8.0	5.4	44
	5	10	8.0	5.4	11
	6	12	8.0	5.2	40
	7	13	8.0	5.0	88
	8	14	7.6	5.1	104
	9	15	7.6	5.2	165 (division stages noted).
	10	16	7.6	5.2	225
	11	17	7.6	5.2	150
	12	18	7.6	5.4	200
	13	19	7.6	5.4	110
	14	20	7.6	5.4	210
	15	21	7.6	5.4	410
	16	22	7.6	5.4	325
	17	23	7.6	5.4	165
	18	24	7.6	5.4	375

could not be accurately estimated during the later hours, since many hundreds of amoebae were agglomerated into large putty-like clumps. In the No. 38 cultures they were fewer in number, reaching a maximum at the fifteenth hour, after which they entirely disappeared. The L.A. medium, however, is not favorable to continued growth on account of the lack of a substrate.

In examining the results of cultures No. 13 and No. 38 in L.E.A. medium (part II in the tables), the lowest pH for No. 38 is reached between the thirteenth and fourteenth hours, and for No. 13 at about the sixteenth hour. In both cases the observation at the close of the second hour shows a larger number of amoebae than in the 5 or 6 succeeding observations. The amoebae are probably those originally inoculated and many may die before multiplication begins. In both the cultures the first decided increase in amoebae is between the thirteenth and fourteenth hours, while the maximum number is at the twenty-first hour for No. 38 and at the twenty-fourth hour for No. 13. However, if only the 2-hourly counts had been taken, as in the case of No. 13, the maximum number of amoebae would have been observed in the twenty-fourth hour. The actual number of organisms, therefore, is not of so great a significance as the general mass effect. The amoebae had undoubtedly multiplied considerably during the last 10 hours, and it was interesting that several cases of binary fission were observed in No. 38 at the fifteenth hour.

Although there are many variables in this type of experiment in impure cultures, yet one gains the general impression that the growth curve of *E. gingivalis* in artificial media follows that of the bacteria and that division tends to begin at about the eighth to the tenth hour in the L.A. medium and between the thirteenth and fourteenth hours in the L.E.A. medium. From then on to the twenty-fourth hour rapid multiplication occurs if the medium is favorable.

2. Omission of Different Salts from the Locke Solution

Since the amoebae seemed sensitive to slight variations in the fluid medium, the following experiments were performed to determine the effects of omitting different salt ingredients from the Locke's solution.

Four solutions were made, each with the omission of a different salt from the ordinary Locke's formula. After sterilization in the autoclave they were added in 10 c. c. amounts to egg slants containing distilled water instead of the usual Locke's solution. No egg albumen was added, so that the coagulated egg slant was the only source of supply for each omitted salt.

Another set of solutions was also prepared, lacking $NaHCO_3$ and dextrose, but containing physiological amounts of either NaCl, KCl, or $CaCl_2$ in different combinations, as shown in table 7. These were added to tubes of the Locke-free egg slants. An approximately equal number of drops from several active, 24-hour cultures of *Endamoeba gingivalis* were inoculated into both the experimental tubes and into control tubes of L.E.A. medium. One control tube for about every 2 or 3 experimental tubes was the usual routine.

In order to determine the limits for the growth of *E. gingivalis* in different dilutions of each salt, several series of tubes were prepared, containing egg slants and 10 c. c. amounts of Locke's solution from which a different salt was omitted in each series. A definite percentage of the desired salt was then added with a pipette in varying amounts to each tube, as shown in table 7. One series, for example, contained tubes of Locke's solution lacking NaCl. To each tube was

TABLE 7. PART I

OMISSION OF DIFFERENT SALTS FROM LOCKE'S SOLUTION

Percentages of the different salts	Number of amoebae in L E cultures having varying percentages of salts											
	24 hours				48 hours				72 hours			
	$CaCl_2$	NaCl	KCl	$NaHCO_3$	$CaCl_2$	NaCl	KCl	$NaHCO_3$	$CaCl_2$	NaCl	KCl	$NaHCO_3$
001%	160a	21a	42a	115a	420	32	488	680	17	40	620	144
005%	550b	16a	120a	19a	640	36	400	720	120	11	420	480
009%	43b	75b	120b	−b	400	100	320	11	390	15	168	95
01%	352c	87b	144b		440	83	1600		44	72	300	
02%	75c			−b	760			1	220			1
05%	44d	5b	26b	−b	132	50	1000	—	300	720	384	—
09%		2c	200c	−c		160	1520	1		920	92	1
1%	28e	2c	53c	4c	272	120	940	7	60	960	264	12
3%	44e	1000d	7c	−c	125	1700	86	2	192	164	144	—
5%	31f	280d	−d	−d	29	1120	17	—	30	360	5	—
7%	1g	70d	−d	−d	—	340	—	—	—	164	4	—
9%	−h	96e	1d	−d	1	744	1	—	—	376	9	—
1%	3h	24e	−e	−e	—	52	—	—	—	160	—	—
2%	−i	−e	−e	−e	—	—	—	—	—	—	—	—
Medium												
L E A a	27	2400	800	720	324	840	1080	1320	1200	760	170	320
b	560	280	100	560	600	800	1400	2240	30	400	240	340
c	43	20	115	50	800	268	1120	760	800	760	560	960
d	8	800	280	170	62	840	1480	1480	240	320	328	800
e	30	68	68	184	45	264	300	600	560	192	492	1400
f	28				48				1000			
g	320				760				40			
h	35				80				66			
i	120				1200				16			
L.E.	240				320				104			
L.E no $CaCl_2$	180i				216				15			
L.E. no NaCl	27a				100				16			
L.E. no KCl	600a				1200				34			
L.E. no $NaHCO_3$	23a				460				1200			
E.S. NaCl, KCl	5j				130				500			
E.S. $CalCl_2$, KCl	4j				9							
E.S. NaCl, $CaCl_2$	2k				78				175			
E.S. NaCl	600k				800				200			
L.E.A. j	480				2800				800			
L.E.A. k	2000				2800				600			

TABLE 7. PART II

CULTURES WITHOUT EGG SLANTS

Medium	Amoeba count			
	Without egg albumen		With egg albumen	
	24 hours	48 hours	24 hours	48 hours
L. without NaCl	—	—	—	—
L. without KCl	—	—	—	—
L. without $CaCl_2$	—	—	—	—
L. without $NaHCO_3$	—	—	—	—
NaCl, KCl, H_2O	—	—	—	3
$CaCl_2$, KCl, H_2O	—	—	—	—
NaCl, $CaCl_2$, H_2O	—	—	—	20
Locke's solution	—	—	—	—
L.A. filtered			17	2
L.A. unfiltered			328	448
L.E.A. controls:				
a	5	50		
b	50	25		
c	100	60		
d	1200	1600		
e	200	800		

added sufficient 10 per cent NaCl solution to give NaCl percentages ranging from .001 per cent to 2 per cent. Tubes with varying amounts of KCl, $CaCl_2$, and $NaHCO_3$ were prepared in like manner. Numerous control cultures of L.E.A. medium were made at the same time, in order to be sure that active amoebae had been inoculated. The small letters accompanying the amoebae counts in the table refer to the corresponding L.E.A. control tubes for the dilutions of each salt, i.e., the "a" under the $CaCl_2$ column refers to the "a" of the L.E.A. medium under the same column, the "b" to that of the "b" control and so forth.

An additional set of the same solutions was prepared without the egg slant base, one series containing unfiltered egg albumen and the other without any egg, as shown in part II of table 7. Observations were made on all the tubes every 24 hours for about 3 days.

Several interesting points were brought out as a result of these experiments. It is evident that the omission of either NaCl, KCl, $CaCl_2$, or $NaHCO_3$ from the Locke formula did not prevent rapid multiplication of *E. gingivalis* provided the egg slant base was present. If the latter was omitted from any of these solutions, no growth occurred. This was also true for a solution of physiological NaCl, and for the several combinations of NaCl, KCl, and $CaCl_2$ without dextrose or $NaHCO_3$, as shown in the table. If egg albumen was added to the solutions, however, growth was more likely to be maintained, as shown in the tube containing NaCl and KCl, and the one containing NaCl and $CaCl_2$.

That the best growth with these different solutions was obtained in the presence of the egg slant might mean either that the salt deficiency was supplied by the coagulated egg or that the amoebae preferred the solid base upon which to creep. Since there was more likely to be growth in the liquid medium if egg white were added, and since better growth occurred in the unfiltered L.A. medium than in the filtered, it seems as if the organisms were more affected by the condition of the substrate than they were by the salt deficiency.

In comparing the results of the experiments in which varying percentages of $CaCl_2$, NaCl, KCl, and $NaHCO_3$ were added to Locke's solutions lacking respectively one of each of these salts, it is seen that, (1) while 0.02 per cent $CaCl_2$ is the amount ordinarily used in the Locke formula, yet *E. gingivalis* is able to grow and multiply in amounts up to 0.5 per cent, and a few organisms even withstood a 1 per cent solution; (2) the amoebae thrive in all of the weaker NaCl dilutions, showing excellent growth up to a 1 per cent solution, but are killed in 2 per cent NaCl; (3) KCl is less tolerated than the NaCl, since there is excellent growth in the lower salt percentages but death of the amoebae occurs when the KCl becomes stronger, 0.5 per cent being about the upper limit; and (4) *E. gingivalis* is unable to withstand a large amount of $NaHCO_3$, because of the high degree of alkalinity produced. The latter experiment was repeated several times, but the amoebae nearly always died in percentages of $NaHCO_3$ above 0.02 per cent, the physiological amount. However, if 24-hour cultures, negative for amoebae but positive for bacteria, were reinoculated with *E. gingivalis,* amoebic growth usually occurs, showing that the amoebae could grow and multiply when the alkalinity of the solution had been sufficiently reduced by the bacteria.

Summary of results—

1. *E. gingivalis* is capable of multiplying in Locke's solution lacking in NaCl, KCl, $CaCl_2$, or $NaHCO_3$, respectively, provided there is a solid base of coagulated egg in the tube, even though the salt content be deficient.

2. *E. gingivalis* will grow in various combinations of NaCl, KCl, and $CaCl_2$ without dextrose or $NaHCO_3$, but the absence of sodium is more detrimental to continued growth than the absence of either calcium or potassium.

TABLE 8

EXPERIMENT WITH THE ADDITION OF VARYING PERCENTAGES OF 10 PER CENT GLUCOSE TO SUGAR-FREE MEDIUM, USING CULTURE NO. 13

	Time	Medium												Control							
	Jan. 25, 1925	0.01% glucose		0.025% glucose		0.1% glucose		0.25% glucose		0.5% glucose		1% glucose		Glucose free		L. no A.		L.A.		L.E.A.	
		pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count
L.A. Medium	24 hours........	5.6	5000+	7.2	—	7.2	200	8.0	120			8.0	12	7.0	Many						
	48 hours........	5.6	15	7.2	—	5.8	600	8.0	—			8.0	—	6.8	1						
	72 hours........	6.0	—																		
	Feb. 4, 1925																				
	24 hours........	6.0	15	5.4	110	4.8	30	4.8	180	4.8	70	4.4	3	6.4	23	7.8	—	6.0	55		
	48 hours........	6.0	—	5.5	2	4.4	10	4.8	25	4.8	—	4.4	—	6.8	—	7.8	—	6.0	—		
	72 hours........	5.8	—	5.8	—	4.6	—	4.8	—	4.8	—	4.6	—	6.9	—			5.4	—		
L.E.A. Medium	Jan. 25, 1925																				
	24 hours........	5.5	55	5.5	14	5.0	8	4.6	2			4.4	—	5.6	28					5.6	1000+
	48 hours........	5.6	112	5.4	40	5.4	90	4.8	—			4.4	—	5.6	21					5.8	1000
	72 hours........	5.8	15	5.6	50	5.5	800	4.8	—			4.4	—	5.8	8					5.8	35
	Feb. 4, 1925																				
	24 hours........	5.6	4	5.4	—	5.1	20	4.6	2	4.4	—	4.2	—	5.8	8	6.0	59			5.6	25
	48 hours........	6.2	16	5.6	14	5.2	32	4.6	—	4.4	—	4.4	—	6.2	75	6.2	150			5.8	200
	72 hours........	6.2	18	5.6	25	5.4	50	4.6	—	4.6	—	4.4	—	6.2	150	6.2	85			5.8	90
	96 hours........			5.6	2	5.4	17			4.8	—	4.4	—	6.2	22					5.8	6

3. Occasional growth may occur in a fluid medium only, if egg albumen is present, but no growth occurs in the various salt combinations when both the egg albumen and the egg slants are omitted, thus showing the need of a suitable substrate.

4. *E. gingivalis* can withstand a high percentage of calcium in the medium but only a small amount of $NaHCO_3$, since a high alkalinity is produced by the latter. The preference of the amoebae for a somewhat acid medium is thus again demonstrated.

3. ADDITION OF VARYING PERCENTAGES OF 10 PER CENT GLUCOSE TO A SUGAR-FREE MEDIUM

Since it was found that the amount of glucose (0.25 per cent) used by Boeck and Drbohlav (1924) for their medium was not favorable for the growth of *E. gingivalis*, the following experiments were performed to discover the effect of adding definite percentages of glucose to sugar-free media. Sterile 10 per cent glucose was added with a pipette to tubes containing 10 c. c. each of the fluid medium, in amounts sufficient to produce the desired percentage. Tubes containing the routine medium with 0.025 per cent glucose in the Locke's solution were used as controls as well as tubes containing sugar-free Locke's. The egg slants used in the experiments with No. 13 contained the ordinary Locke's solution, while those used with No. 38 were sugar free.

On January 25, 1925, strain No. 13 was inoculated into both L.A. and L.E.A. media, containing the glucose percentages shown in table 8. In the tubes of L.E.A. medium it may be seen that, as the percentage of glucose increases the pH becomes lower, due to the stronger fermentative action of the bacteria in the greater concentration of carbohydrates. The amoebae were found to favor the tubes containing the smaller percentages of glucose, 0.1 per cent being the optimum limit, while little or no growth occurred in the tubes with 0.25 per cent and 1 per cent sugar, respectively, in both of which the pH remained below pH 5.0. Although the organisms grew in the sugar-free medium, yet they evidently prefer a certain small amount for optimum multiplication.

The results of the cultures in L.A. medium were not very uniform, especially since no organisms were found in the tubes containing 0.025 per cent glucose, in which percentage growth ordinarily occurs. For some unknown reason the bacteria evidently did not increase sufficiently to reduce the pII in the tubes containing the higher sugar percentage, so that the amoebae were present in the first 24 hours. They later disappeared. The greatest number of organisms, however, were counted from the sugar-free tubes and from the tubes below 0.25 per cent glucose.

The same experiments with strain No. 13 were repeated on February 4, using the same sugar-free Locke's solution. A third set of control tubes containing Locke's solution (0.025 per cent glucose) and without albumen were added to the series and also tubes with 0.5 per cent glucose. Results were obtained in the L.E.A. cultures comparable to those obtained on January 25. There was the same increase in acidity with the increase in sugar concentration, and also the same inability of the amoebae to survive in glucose percentages above 0.1 per cent. This percentage is favorable but beyond it no growth is sustained. Favorable results were also obtained in both the sugar free and the L.A. medium and in the Locke-egg slant cultures without albumen.

Amoebae were found in all of the L.A. tubes in 24 hours, after which they soon disappeared. There seems to be a greater buffer action in the Locke-albumen fluid without the egg slants, so that the organisms tend to survive a longer time in the higher sugar concentrations. They soon die off, however. No amoebae were found in the Locke's solution without the albumen, thus showing the need for albuminous strands upon which they may creep.

The same experiment was again repeated on March 24, using strain No. 38 instead of No. 13. Since egg albumen itself contains a small amount of glucose, one set of tubes was prepared with Locke's solution without albumen. No cultures were run without egg slants.

The results from both series of tubes as shown in table 9 were entirely comparable to those obtained from the L.E.A. tubes of strain No. 13. There was the same decrease of pII with increase of sugar concentration and the same lack of growth in the glucose concentration above 0.1 per cent. Amoebae were also found to multiply in sugar-free media, with and without slants.

4. Experiments with Lipoids

In many of the cultures containing the egg slant medium, numbers of amoebae were often found to have ingested certain round refractile ("shiny") bodies of different sizes. Sometimes only one large inclusion would be noticed, having a slightly irregular border with a double outline like that of a protozoan cyst wall. At other times, especially in older cultures, the amoebae would be filled with as many as 20–30 of these inclusions, smaller in size. Numerous bodies having the same morphological appearance were also noticed free in the surrounding medium and also in the flakes of material from the egg slant.

TABLE 9

EXPERIMENTS WITH THE ADDITION OF VARYING PERCENTAGES OF 10 PER CENT GLUCOSE TO SUGAR-FREE MEDIUM, USING CULTURE NO. 38

Initial pH for all tubes = pH 7.8

I. *L.E. Medium. Planted March 24, 1925

Time	Medium												Controls			
	0.01% glucose		0 025% glucose		0 1% glucose		0 25% glucose		0 5% glucose		1% glucose		Glucose free		L.E.	
	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count
24 hours	6 2	12	5 8	8	4 6	1	4 6	1	4 6	—	4 4	—	6 6	1	6 6	1
48 hours	6 4	238	6 4	140	5 4	11	4 8	1	4 8	—	4 8	—	6 6	75	6 6	5
72 hours	6 4	30	6 8	79	5 4	77	5 0	—	4 8	—	4 8	—	6 6	65	6 6	12

II. *L.E.A. Medium. Planted March 24, 1925

Time	Medium												Controls			
	0 01% glucose		0 025% glucose		0 1% glucose		0 25% glucose		0 5% glucose		1% glucose		Glucose free		L E.A.	
	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count
24 hours	5 6	9	5 4	14	5 0	1	4 8	—	4 6	—	4 4	—	6 2	14	5 4	4
48 hours	6 2	24	5 8	130	5 2	40	4 8	—	4 8	—	4 6	—	6 2	33	5 8	18
72 hours	6 0	71	5 6	147	5 4	245	4 8	—	4 8	—	4 6	—	6 2	83	5 6	113
96 hours			5 6	90												

* All egg slants were glucose free except controls L.E. and L.E.A. which contained the ordinary amount of glucose.

Scrapings from the slants of uninoculated tubes also showed the same type of body, so that they evidently were constituents of the egg yolk.

The bodies did not stain with iodine, so apparently they were not glycogen. When Sudan III stain was applied to the fresh slide preparation, however, both the bodies in the amoebae and those outside stained a deep red color, showing their lipoidal nature. But when slides were stained by the iron-haematoxylin method, the amoebae were often noticed to contain varying numbers of spherical inclusions having a very faint grayish hue (pl. 12, fig. 18) and surrounded by a wide clear area. Occasionally only a single large body would be found in an amoeba and one could notice the irregular outer border characteristic of the unstained forms. It seems reasonable to believe that the bodies noticed in the amoebae in both the stained and the unstained material were of the same origin. It was difficult, however, to reconcile the fact that the same body could both take up a lipoidal stain, like Sudan III, and also show a slight reaction with the iron-haematoxylin method. Usually fatty substances are dissolved out in the latter process and do not stain. Since the haematoxylin stained inclusions always had a large clear area around them and since egg yolk (Hawk, 1923) may contain substances composed of lecithin-protein mixtures, it seems conceivable that these bodies may be of that nature. Further analysis of their chemical composition should be made to prove this point. There is no doubt, however, that they are of yolk origin and that they contain a lipoidal constituent.

Because of the preference of *E. gingivalis* for these lipoidal bodies, the following experiments were performed to discover their reaction to the presence of a liquid, organic lipoid in the medium. Cod liver oil was added in the percentages shown in table 10 to 10 c. c. amounts of both plain Locke's solution and the Locke-albumen medium with egg slants. The oil did not mix easily with the solutions but rose as a thin layer on the surface of the liquid. A better emulsion, however, was secured with the Locke-egg albumen solution than with the plain Locke's.

Excellent growth occurred in all the oil dilutions in both series of cultures containing the egg slants. The counts were higher and the amoebae remained viable for a longer period in the tubes containing the oil than in the control tubes. Whether this effect was due to the utilization of the oil by the amoebae; to the lowering of the surface tension by the presence of the oil; or to a decrease in the oxygen tension by the lipoidal surface film, it is difficult to say. Refractile inclusions were noticed in many of the amoebae in the oil cultures, but it is a matter of conjecture whether or not any of them were due to the assimilation of the lipoid, since they were also present in the control tube.

In an attempt to determine this point, experiments were undertaken as shown in table 11. Tubes were used containing, respectively, slants made of the egg white alone and of the egg yolk alone, covered with either plain Locke's or with Locke-albumen solution. 0.1 c. c. of

TABLE 10

EXPERIMENT IN THE ADDITION OF VARYING AMOUNTS OF COD LIVER OIL TO THE LIQUID MEDIUM, USING CULTURE NO. 13

I. L.E. Medium. Planted February 6, 1925

Initial pH of L.E. with oil = pH 7.0; without oil = pH 7.4

Time	Medium												Control		
	0.01% oil			0.1% oil			0.25% oil			0.5% oil			L.E., no oil		
	pH	Amoeba count	Amoeba count with shiny bodies	pH	Amoeba count	Amoeba count with shiny bodies	pH	Amoeba count	Amoeba count with shiny bodies	pH	Amoeba count	Amoeba count with shiny bodies	pH	Amoeba count	Amoeba count with shiny bodies
24 hours.	5 4	180	—	5 5	61	27	5 5	160	69	5 4	24	7	5 4	112	—
48 hours.	6 2	160	38	6 2	119	14	6 2	275	50	5 8	145	40	6 2	—	—
72 hours.	6 0	164	23	6 2	125	30	6 2	210	40	6 0	52	11	6 2	—	—
96 hours.	6 0	20	—	6 0	11	—	6 0	20	—	6 0	15	—	6 2	—	—

II. L.E.A. Medium. Planted February 6, 1925

Initial pH of L.E.A. with oil = pH 7.6; without oil = pH 7.8

Time	Medium												Control		
	0.01% oil			0.1% oil			0.25% oil			0.5% oil			L.E.A., no oil		
	pH	Amoeba count	Amoeba count with shiny bodies	pH	Amoeba count	Amoeba count with shiny bodies	pH	Amoeba count	Amoeba count with shiny bodies	pH	Amoeba count	Amoeba count with shiny bodies	pH	Amoeba count	Amoeba count with shiny bodies
24 hours.	5 4	15	7	5 6	67	12	5 4	16	4	5 3	19	4	5 6	10	—
48 hours.	5 8	60	10	5 8	245	112	5 6	32	9	5 6	36	4	5 8	19	4
72 hours.	5 6	67	16	5 6	220	65	5 6	143	40	5 8	65	17	6 2	51	13
96 hours.	5 6	27	4	5 6	27	—	5 6	37	—	5 8	61	—	6 2	30	3

cod liver oil containing Sudan III was added to 10 c. c. of each fluid, except the controls, in the hope that the dye might show the ingestion of any stained oil globules. Amoebae were present in all the tubes but no very conclusive results were obtained in respect to oil ingestion. Some of the amoebae, however, seemed to have large, unusually pink vacuoles. The stained oil remained largely on the surface of the fluid and very little was noticed in the bacterial sediment. The chief point of interest to be noted in this experiment was that, while growth was obtained in both the separate egg white slant and egg yolk slant media, yet the whole-egg medium seemed the most favorable, especially in the tubes of Locke's without the albumen.

TABLE 11

EXPERIMENTS WITH CULTURE NO. 13, USING EGG WHITE AND EGG YOLK SLANTS WITH AND WITHOUT THE ADDITION OF 0.1 PER CENT COD LIVER OIL CONTAINING SUDAN III

I. Locke's solution used as the fluid medium. Planted February 16, 1925

Initial pH of Locke's = pH 7.8; of L.A. = pH 8.2

Time	Medium																	
	Egg yolk, oil, Sudan III			Egg yolk, no oil			Egg white, oil, Sudan III			Egg white, no oil		Whole egg, oil, Sudan III			L.E., no oil		
Hours	pH	Amoeba count	Amoeba with shiny bodies or dye	pH	Amoeba count	Amoeba with shiny bodies	pH	Amoeba count	Amoeba with shiny bodies or dye	pH	Amoeba count	pH	Amoeba count	Amoeba with shiny bodies or dye	pH	Amoeba count	Amoeba with shiny bodies
24	5.5	10	1	5.5	45	No cell inclusions.	6.2	30	4, one with slight pink tinge.	7.2	37	6.0	114	5 not pink	6.0	87	......
48	6.2	4	......	6.0	8	......	6.8	26	......	6.8	25	6.2	117	......	6.4	110	12
72	......	......	......	6.0	—	......	7.0	—	......	......	......	......	......	......	6.6	90	......

II. L.A. used as fluid medium. Planted February 16, 1925

Time	Medium																	
	Egg yolk, oil, Sudan III			Egg yolk, no oil			Egg white, oil, Sudan III			Egg white, no oil		Whole egg, oil, Sudan III			L.E.A., no oil		
Hours	pH	Amoeba count	Amoeba with shiny bodies	pH	Amoeba count	Amoeba with shiny bodies	pH	Amoeba count	Amoeba with shiny bodies or dye	pH	Amoeba count	pH	Amoeba count	Amoeba with shiny bodies	pH	Amoeba count	Amoeba with shiny bodies
24	5.5	60	6—2 with pink vacuoles.	5.3	6	......	6.0	16	4 with pink vacuoles.	6.1	325	6.0	35	6 not stained	5.8	95	......
48	5.7	16	......	6.0	7	......	6.4	46	......	6.4	177	6.2	170	25 not stained	6.0	270	......
72	6.0	19	......	......	......	......	......	......	......	6.4	65	......	......	......	6.0	80	......

Since the cod liver oil-Sudan III mixture usually remained as a surface film on the liquid medium, these substances were added directly to egg-white slants. In the coagulated medium the stained material was found on the surface of the slant. Excellent growth was obtained in this medium with the addition of the L.A. solution, but no stained ingested particles were noticed. Some organisms, however, contained the unstained yolk bodies, probably derived from small pieces of yolk carried over in the inoculation.

The effect of the addition of a 10 per cent ethereal solution of cholesterol to the different media was also tried. Negative results were obtained in the cholesterinized series containing Locke's solution without albumen, with and without egg slants, while the control L.E. tube showed amoebae to be present. In the L.A. series, growth in the cholesterinized tubes was obtained in the egg slant cultures but not in the others. The amoebae evidently are able to endure small amounts of cholesterol when egg albumen is also present, but are unable to in its absence.

Summary of results—

1. *E. gingivalis* is able to ingest lipoidal bodies contained in egg yolk. Although excellent growth may be obtained from the addition of a liquid lipoid, such as cod liver oil, yet no very definite evidence has been secured in regard to its assimilation by the organisms.

2. Growth may occur in media containing slants either of egg yolk or of egg white alone, but better results are secured by using the whole egg. Slight growth may also occur with the addition of cholesterol to L.E.A. medium.

5. Experiments in the Addition of Protein and Amino Acids to the Different Media

(1) *Locke-Lewis medium*—

Table 12 shows the effect of inoculating *E. gingivalis* into tubes containing a modified Locke-Lewis medium (Lewis and Felton, 1922), using ordinary beef infusion broth instead of chicken broth. With this medium 15 c. c. of broth are added to 85 c. c. of Locke's solution containing 0.25 per cent glucose. Since this amount of glucose was detrimental to the gowth of the amoebae, tubes of Locke's and broth without the sugar were also used.

TABLE 12

THE EFFECT OF USING INFUSION BROTH IN CULTURE NO. 13

Planted January 13, 1925

Time	Medium												Control	
Hours	Locke, broth and 0.25% glucose		Locke, broth		Broth, no Locke's		L.E broth and 0.25% glucose		L E broth		E. broth, no Locke's		L.E A., no broth	
	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count
Initial pH	7 4	—	7 2	—	7 0	—	7 4	—	7 4	—	7 0	—	8 0	—
24	4 4	—	6 2	3	6 0	—	4 4	—	5 8	4	6 0	—	5 6	10
48	4 4	—	7 4	1	7 1	—	4 4	—	6 2	30	6 8	2	5 8	86
72		—	8 0	—		—		—	6 8	6	7 4	—	5 8	25

Practically no growth was observed in tubes of broth undiluted with Locke's solution, while negative results were also found in the cultures with 0.25 per cent glucose. Amoebae were present, however, in the Locke-Lewis medium both with and without egg slants. The latter secured more favorable growth, although the counts did not equal those found in the control tube. It may be noted, therefore, that, while infusion broth is too concentrated a fluid for amoebic growth, growth may occur in a dilute broth solution such as is found in the Locke-Lewis medium.

(2) *Peptone experiments*—

The effect of adding different percentages of sterile 10 per cent peptone solution to L.E. and L.E.A. media is shown by table 13. Growth was obtained in every instance, although the amoebae in the L.E. cultures were smaller and more sluggish than those from the L.E.A. medium. This may have been due, however, to the higher degree of pH obtained in the former tubes. As the acidity declined the amoebae tended to die off. The percentage of peptone did not seem to particularly affect the amoebic growth, though the largest numbers were found in the 24-hour L.E. medium with a 1 per cent concentration.

(3) *Cystin experiments*—

Several experiments were made with the addition of 0.1 per cent cystin solution to the different media. Since cystin is not readily soluble except in a strongly acid solution, this amino acid tended to crystallize out in the culture tubes. For this reason most of the experiments were somewhat unsatisfactory.

TABLE 13

Experiments in the Addition of Peptone to Cultures of No. 13

I. L.E. Medium. Initial pH = pH 7.6. Planted February 28, 1925

Time	Variations in amount of Peptone									Control	
	0.1% Peptone			0.5% Peptone			1% Peptone			L.E.	
Hours	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count
24	6.4	21	Amoebae sluggish	6.6	36	Amoebae small, sluggish	6.6	140	Amoebae sluggish, thickened up	6.5	26
48	6.6	11	Small	6.6	15	Small, more active	6.8	8	Small, degenerate amoebae	6.6	18
72	6.6	1		6.6	4	Very small	6.8	—		6.6	2

II. L.E.A. Medium. Initial pH = pH 8.0. Planted February 28, 1925

Time	Variations in the amount of Peptone									Control	
	0.1% Peptone			0.5% Peptone			1% Peptone			L.E.A.	
Hours	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count
24	6.2	53	Amoebae active, rather large	6.2	14	Amoebae active	6.4	45	Amoebae active	6.0	129
48	6.0	78	Large, active, granules	6.4	9		6.6	17		6.0	101
72	6.0	2		6.4	4		6.6	5		6.0	13

TABLE 14
THE EFFECT OF CULTURING NO. 13 WITH CYSTIN

I. Planted November 24, 1924

Time	Medium				Control	
Hours	L.A. and 0.1% cystin		L.E.A. and 0.1% cystin		L.E.A., no cystin	
	pH	Amoebae count	pH	Amoebae count	pH	Amoebae count
Initial pH	7.7	...	7.7	...	8.2	...
24	6.6	17—active	5.6	8	5.6	22
48	5.4	250—amoebae large, active	5.6	77	5.4	715
72	5.4	—	5.6	—	5.8	—

Inoculations made directly from the mouth into the same media as above

No. 26. Planted November 24, 1924

Time	Medium				Control	
Hours	L.A. and 0.1% cystin		L.E.A. and 0.1% cystin		L.E.A., no cystin	
	pH	Amoebae count	pH	Amoebae count	pH	Amoebae count
Initial pH	7.7	...	7.7	...	8.2	...
24	6.6	1	5.4	—	5.2	1
48	6.0	250 in clumps of debris	5.8	12	5.5	44
72	6.0	—	...	...	...	—

Summary of results—

1. There was favorable growth of amoebae in L.A. and L.E.A. cultures plus 0.1 per cent cystin when inoculations were made both directly from the mouth and from culture No. 13.

2. There was favorable growth of strain No. 13 in cystin, Locke-Lewis media.

3. The presence of amoebae in cystin-Locke's solution without egg slants was an unusual occurrence as amoebae were not found at any other time in the plain Locke's fluid (table 14). While no very remarkable results were obtained by the addition of this amino acid, yet apparently it is not detrimental to the life of *E. gingivalis* and may even favor growth in some instances.

(4) *Tyrosin experiments*—

Experiments with the addition of tyrosin to the cultures were made in the same manner as those with cystin. These results, also (table 15), were rather unsatisfactory on account of the insolubility of the amino acid and its tendency to precipitate out in the tubes. Growth, however, was obtained in a few cultures in the L.E. medium with

0.01 per cent tyrosin, and in the L.E.A. tube with 0.1 per cent tyrosin. It is uncertain, therefore, whether or not this amino acid would have the same "auxetic" effect upon the growth of *E. gingivalis* as Cropper and Drew (1914) found it to have for certain free-living forms of amoebae.

TABLE 15

EXPERIMENT IN THE ADDITION OF TYROSIN TO CULTURES OF NO. 13

I. Locke's Medium. Initial pH = pH 7.8. Planted February 9, 1925

Time	Medium								Controls			
Hours	L. and 0.01% tyrosin		L. and 0.1% tyrosin		L.E. and 0.01% tyrosin		L.E. and 0.1% tyrosin		Locke's, no tyrosin		L.E., no tyrosin	
	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count
Initial pH	7.2	—	7.2	—	7.2	—	7.2	—	7.8	—	7.8	—
24	6.6	—	7.2	1	5.9	32	5.5	—	7.0	—	5.6	1
48	6.6	—	7.4	—	6.4	12	6.4	—	7.0	—	6.2	62
72					6.4	33	6.4	4			6.4	21

II. L.A. Medium. Initial pH = pH 7.4. Planted February 9, 1925

L.E.A. Medium. Initial pH = pH 7.4 Planted February 9, 1925

Time	Medium								Controls			
Hours	L.A. and 0.01% tyrosin		L.A. and 0.1% tyrosin		L.E.A. and 0.01% tyrosin		L.E.A. and 0.1% tyrosin		L.A., no tyrosin		L.E.A., no tyrosin	
	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count	pH	Amoeba count
Initial pH	7.2		7.0		7.2		6.8		7.4		7.4	
24	5.5	—	5.4	—	5.4	—	5.4	—	5.9	1	5.6	2
48	5.5	—	5.4	—	6.0	1	6.0	—	5.5	2	6.0	18
72		—			5.6	—	6.4	24	5.5	30	5.8	14

6. EXPERIMENTS IN THE ADDITION OF BLOOD TO CULTURES OF E. GINGIVALIS

Since there has been much controversy as to whether or not *E. gingivalis* ingests red blood corpuscles and therefore might act as a tissue destroyer like *E. dysenteriae*, the following experiments were performed with the addition of both rabbit and human blood to cultures, No. 38 and No. 13, respectively.

(1) *Strain No. 38 with the addition of human blood*—

Eight c.c. of human blood were removed aseptically from the median basilic vein of the arm. Half of the blood was allowed to clot in a sterile centrifuge tube and the other half was defibrinated by

TABLE 16

EXPERIMENTS IN THE ADDITION OF HUMAN BLOOD TO CULTURE NO. 38

I. L.E. Medium. Planted March 13, 1925. Initial pH = pH 7.8

Time	Variations of the blood added to L.E medium															Control	
	0.05 c. c. human washed RBC			0.2 c. c. hemolysed human washed RBC			0.05 c. c whole human blood			0.2 c c. hemolysed whole human blood			0.3 c. c. human blood serum			L.E.	
Hours	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count
24	6.0	40	1 with ingested RBC.	6.0	24	Amoebae large.	6.2	1					6.0			6.2	134
48	6.2	274	70 with RBC inside.	6.4	50	Amoebae large.	6.2	17	1 with ingested RBC		55		6.4	4		6.3	125
72	6.2	71	13 with RBC	6.4	45	Amoebae large.	6.2	18			107	Amoebae with vacuoles, elongated	6.2	15	Large	6.4	63

II. L.E.A. Medium. Planted March 13, 1925. Initial pH = pH 7.4

Time	Variations of the blood added to L E. medium															Control	
	0.05 c. c. human washed RBC			0.2 c. c. hemolysed human washed RBC			0.05 c. c whole human blood			0.2 c. c. hemolysed whole human blood			0.3 c. c. human blood serum			L.E.A.	
Hours	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count
24	5.5	150	No ingested RBC.	5.5	12		5.6	60	No ingested RBC.		1		5.6	37		5.6	88
48	6.0	95	3 amoebae with RBC.	6.0	200	Remains of hemolysed RBC in a few amoebae.	5.8	50			22	Large amoebae.	6.0	24	Granular Amoebae.	6.0	400
72	6.0	40	8 with RBC hemolysed	6.0	66		5.8	40			17	Large amoebae	6.0	47	Large granules.	6.0	256

shaking with glass beads in a small sterile flask. About 2 c. c. of the defibrinated blood were centrifugalized and washed three times with sterile physiological saline. Small amounts of both the washed cells and of the whole blood, respectively, were then diluted one part to three parts with sterile water, to hemolyze the red blood cells.

Hemolyzed and non-hemolyzed washed red blood cells; hemolyzed and non-hemolyzed whole blood; and also undiluted serum were added to tubes of L.E. and L.E.A. medium, respectively, in the amounts shown in table 16. The tubes were warmed and then inoculated with 4 drops each of material from strain No. 38. Control tubes without blood were also inoculated. Observations were made every 24 hours for 3 successive days.

In comparing the amount of growth in the different experimental cultures, it was observed that while there were amoebae in all the tubes, the most favorable results were obtained in the cultures containing the washed red blood cells, both hemolyzed and unhemolyzed. Their numbers, however, did not equal those in the control tubes.

The most interesting result of this experiment was the finding of amoebae with ingested red blood corpuscles in the tubes containing the washed human cells. A 24-hour observation showed one amoeba with ingested red blood corpuscles in the L.E. culture. None were noticed in that of the L.E.A. medium, but in 48 hours the former (L.E.) tube contained large numbers of amoebae, many of them with red blood cells in various stages of preservation. Of the 274 amoebae counted, 70 possessed from one to six included erythrocytes.

These cells had the same brassy color and the biconcave appearance of the red blood corpuscles found free in the surrounding medium, or else they were crenated and degenerated in the same fashion as the exterior red cells. The included cells were seen to move about with the flowing endoplasm of the amoeba, showing their color and biconcavity in such a manner as to leave no doubt as to their identity. Wright's stained slides showed typical pink erythrocytes within the blue endoplasm of the amoeba. The 48-hour L.E.A. culture, containing washed human cells, also showed amoebae with ingested red blood corpuscles. They were not so numerous as in the other culture tube.

(2) *Strain No. 38 with addition of rabbit's blood*—

Table 17 gives the results of the addition of whole and of washed rabbit's blood to cultures of No. 38. Unusually fine growth was obtained in all of the cultures but the number of amoebae with ingested red blood cells was very much less in proportion to the total counts than was the case when using human blood. Whether or not this meant any species differentiation on the part of the amoebae with respect to the kind of red cells ingested, it is hard to say.

TABLE 17

EXPERIMENTS IN THE ADDITION OF RABBIT'S BLOOD TO CULTURE NO. 38

Initial pH of L.E. Medium = pH 7.8. Planted March 16, 1925

Initial pH of L.E.A. Medium = pH 7.6. Planted March 16, 1925

Time	Medium									Control	
	L.E. and 0.05 c. c. washed rabbit's RBC			L.E. and 0.05 c. c. whole rabbit's RBC			L.E.A. and 0.05 c. c. washed rabbit's RBC			L.E.	
Hours	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count
24	6 2	70	1 with a hemolyzed RBC.	6 2	42	No ingested RBC.	5 6	43	No ingested RBC.	5 4	46
48	6.4	315	2 amoebae with crenated RBC	6 4	500	Amoebae large, no recognized ingested RBC	6 4	394	14, with small crenated RBC	6 2	1200
72	6 6	50	No ingested RBC	6.6	36	1 with a hemolyzed RBC	6 4	43	2 with small RBC	6 2	180

Besides these test tube experiments, observations were also made on the behavior of *E. gingivalis* when washed red blood cells were added directly to a slide preparation on a warm stage. The amoebae flowed over, under, and in between the corpuscles, but no actual ingestion of either human or animal cells was ever observed. The red cells, on the other hand, were often found adhering in small clumps to the trailing posterior end of an organism or else were adherent to the dorsal surface. The animal seemed to have the ability to carry along as many as 20 to 30 of these cells at one time.

The apparent lytic property of the amoebae was noted on two separate occasions; once when washed human cells were added to a slide preparation of strain No. 38, and again in a preparation from a culture of No. 13 with washed rabbit red blood cells. In both observations several normal erythrocytes were plainly visible close to an amoeba. Within five minutes, however, these cells completely faded from view, except for a faint outline, while the erythrocytes in the surrounding medium remained apparently intact. This phenomenon was watched with much interest, as it opens the question in regard to the enzymic action of the amoebae. Do they have the ability to both ingest whole red blood cells, dissolving them internally, and also to secrete an enzyme which will destroy the corpuscles before ingestion? The exercise of the latter property might account for much tissue destruction without any visible signs of red blood cell ingestion being observed within the amoebae. The necessity for a bacteria free culture of amoebae to prove this point is thus demonstrated.

TABLE 18

Experiments in the Addition of Human and of Rabbit's Blood to Culture No. 13

I. L.E. Medium. Initial pH = pH 7.8. Planted March 16, 1925

Time	Variations of the blood added to the medium								
	L.E. and 0.05 c.c. washed rabbit's RBC			L.E. and 0.05 c.c. of whole rabbit's blood			L.E. and human washed RBC		
Hours	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks
24	6.2	10	No ingested RBC, watched an amoeba dissolve several RBC	6.2		No ingested RBC	6.4	4	No ingested RBC RBC too thick
48	6.4	29	No ingested RBC	6.4	12			11	No ingested RBC
72	6.6	3	No ingested RBC	6.6	3				

II. L.E.A. Medium. Initial pH = pH 7.8. Planted March 16, 1925

Time	Variations of the blood added to the medium						Control		
	L.E.A. and 0.05 c.c. washed rabbit's RBC			L.E.A. and whole rabbit's RBC			L.E.A.		
Hours	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks	pH	Amoeba count	Remarks
24	5.8	32	No ingested RBC	5.8	7	No ingested RBC	5.8	35	Amoebae small
48	6.0	17	Large, granular amoebae, no ingested RBC	6.0	5		6.0	56	Amoebae granular
72	6.2	63	No ingested RBC	6.2	9		6.2	15	Amoebae elongated

(3) *Strain No. 13 with addition of both human and rabbit blood*—

Table 18 shows the results of adding both human and rabbit blood, respectively, to cultures of strain No. 13, using the same methods as described above. Although lysis of the erythrocytes was noticed on the one occasion mentioned, yet no ingested red blood cells were found in the amoebae fom any of these cultures. Unrecognizable red cell remains, however, might have been present in a few organisms, since many were very granular.

Summary of results—

1. *E. gingivalis* is capable of ingesting both human and rabbit erythrocytes.
2. Although *E. gingivalis* grows well when different variations of both human and rabbit blood are added to the cultures, yet the growth is actually no more abundant than when the customary L.E.A. medium is used.

VI. THE EFFECT OF LOW TEMPERATURES UPON THE GROWTH OF E. GINGIVALIS

In order to determine the viability of *E. gingivalis* at temperatures below 37° C., several experiments were performed on three separate strains. The first three tubes of table 19 were inoculated with strain No. 13 on March 2, 1925, at 3:30 P.M. Tube No. 1 was placed in the incubator at 37° C., tube No. 2 was left at room temperature, and tube No. 3 was placed outdoors in a northern exposure. The tubes were all examined in 24 hours with negative results except for tube No. 1. Tubes No. 2 and No. 3 were then placed in the incubator overnight. No growth occurred in No. 3. Seven amoebae were found in 24 hours and 4 in 48 hours in tube 2 at 37° C.

In tube No. 4 the culture was first started at 37° C., showing 14 amoebae in 24 hours, and was then placed outdoors. It was examined on the following day with negative results, but after a 24-hour period in the incubator, 8 motile amoebae were found. In subsequent exposure to the exterior temperature no amoebae appeared.

The same course of procedure was carried out for strains No. 38 and No. 42, respectively, although the experiment with the latter was incomplete. As a result of these temperature experiments it was found that while the best and most active growth was obtained at

37° C., yet the three different strains were all able to survive the exposure to room temperature (R.T.) for at least 24 hours if not 48 hours, not only when initial growth had been secured by a primary inoculation at 37° C. but when the freshly inoculated culture was left in the room without the primary incubation. The amoebae in these instances were very sluggish, thickened up, and almost cyst-like, but could be revived to motility by placing them on the warm stage. Exposure to the outdoor temperatures usually killed the amoebae, although a very few organisms were found on two occasions after this exposure and a subsequent incubation at 37° C.

TABLE 19

EFFECT OF LOWER TEMPERATURES THAN 37° C. UPON THE GROWTH OF ENDAMOEBA GINGIVALIS

Culture No. 13

Planted					Examined		
Tube No.	Date	Time	Medium	Temperature	Date	Time	Amoeba count
1	3/2/25	3:30 P.M.	L.E.A.	37° C.	3/3/25	3:35 P M.	21
				37° C.	3/5/25	5:00 P.M.	—
2	3/2/25	3:30 P M.	L.E A.	R T	3/3/25	3:30 P M.	
				37° C.	3/5/25	7:45 P.M.	7, very sluggish
				R T.	3/6/25	4:45 P.M.	4, small
				R T.	3/7/25	11:45 A.M.	—
				37° C.	3/9/25	10:45 A.M.	—
3	3/2/25	3:30 P.M.	L E A.	Outdoor temp.	3/3/25	3:25 P.M.	—
				37° C.	3/5/25	7:30 P.M.	—
4	3/2/25	5:30 P M.	L E.A	37° C.	1/16/25	10:00 P.M.	14
				Outdoor temp. 16° C	1/17/25	8:30 P.M.	—
				37° C.	1/18/25	8:00 P.M.	8, motile
				Outdoor	1/21/25	3:25 P.M	—
				temp. 37° C.	1/22/25	4:00 P.M.	—

This ability of *E. gingivalis* to withstand a reduction of temperature below that of the body is of interest when one considers the habitat of the organism. Undoubtedly the amoebae in the mouth are often exposed to sudden variations in temperature and have therefore acquired the ability to withstand these changes. Further study should be undertaken to determine the limits of growth in temperatures above 37° C.

VII. SUMMARY

Although bacteria-free cultures were not used in this work, yet certain general conclusions may be drawn that may aid toward a better understanding of the relationship of this organism to its habitat in the human mouth. The actual metabolic activities and their connection with pathogenic processes can be learned, however, only through work on a pure culture of the amoebae. It is unknown whether or not amoebae would grow in a medium free from bacteria, but if such a pure culture be ever attained, an entirely new field of experimentation would lie open to the protozoologist.

The main points of interest learned from this work on *Endamoeba gingivalis,* in spite of the variable factors, may be summarized in the following general review.

1. It has been found possible to grow *E. gingivalis* in an artificial culture medium and there seems no reason to doubt that such a culture can be prolonged indefinitely if proper care be maintained. The most favorable medium for continued cultivation is a modification of the Locke-egg slant-albumen medium of Boeck and Drbohlav (1924).

2. The growth curve of the amoebae in mixed bacterial culture follows that of the bacterial curve: increase in growth to a maximum and a gradual decline when the food is exhausted and the waste products accumulate. The curve may be maintained at a more constant level, however, if fresh liquid be added.

3. Although *E. gingivalis* may survive and apparently multiply in media with an initial wide range of hydrogen-ion concentration, the initial pH for optimum growth lies between pH 7.0 and pH 7.8. No matter in which hydrogen-ion concentration the organisms are first planted, the bacteria tend to bring the reaction to a certain general level, around pH 5.4–6.2. If there is any increase in proteolytic or putrefactive processes because of the presence of flagellates or unfavorable bacteria, the amoebae rapidly degenerate.

4. *E. gingivalis* is apparently unaffected by the omission of either NaCl, $CaCl_2$, KCl, or $NaHCO_3$, respectively, from the Locke's solution, or by various combinations of NaCl, $CaCl_2$, or KCl without dextrose or $NaHCO_3$, provided a solid base of coagulated egg is present. The amoebae will not grow in any solution of salts which is lacking in

either the coagulated egg or the egg albumen. They can apparently withstand a high percentage of calcium but not of $NaHCO_3$ and appear to be slightly more affected by omissions in the sodium content than by those in the potassium or the calcium content.

5. While the amoebae will grow in a sugar-free medium, the optimum growth is obtained in glucose concentrations as high as 0.1 per cent. If the concentration is above that amount the organisms soon disappear. This is probably due to the continued maintenance of a very low pH by the fermentative bacteria.

6. *E: gingivalis* feeds on bacteria in the cultures and also shows a strong preference for the ingestion of certain lipoidal yolk bodies of unknown nature.

7. Although the amoebae may show excellent growth in the presence of a liquid fat, like cod liver oil, there is no observed evidence of its assimilation.

8. *E. gingivalis* will grow in a dilute broth medium, and in different media to which peptone, cystin, and tyrosin, respectively, have been added.

9. Ingested red blood corpuscles, both of human and of animal origin, have been found in *E. gingivalis*, although there is a greater preference for the washed human erythrocytes than for the others.

10. *E. gingivalis* is able to exist at temperatures below 37° C., but the latter is the most favorable for optimum growth.

11. Finally, on the whole *E. gingivalis* in mixed bacterial cultures is able to withstand a wide range of hydrogen-ion concentration; is not easily influenced by disturbances in the normal salt balance; is able to ingest lipoidal yolk bodies and both human and animal red blood corpuscles, respectively; and is able to exist and multiply in the presence of cod liver oil, of glucose in small concentrations—not above 0.1 per cent—and of certain proteins, peptones, and amino acids.

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EXPLANATION OF PLATES

PLATE 10

All figures drawn from preparations stained by the iron haematoxylin method. Camera lucida drawings. × 2500.

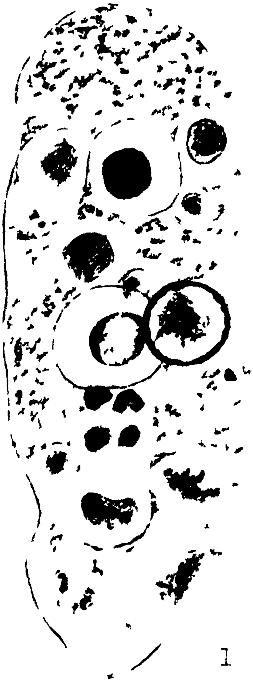
Fig. 1. *E. gingivalis* from a direct smear from the gingival exudate of case 38. The large food inclusions are shown.

Fig. 2. *E. gingivalis* from a culture of 38. Note the same type of karyosome as in figure 1.

Fig. 3. Amoeba from a direct smear from the mouth of case 40.

Fig. 4. Amoeba from a gingival smear of case 13. Note the cell inclusions.

Fig. 5. Amoeba from a L.E.A. culture of strain No. 13. Same type of karyosome as seen in figure 4.



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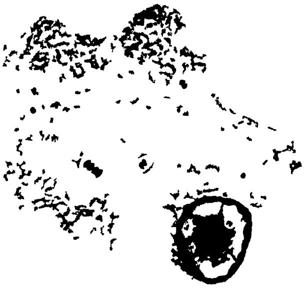

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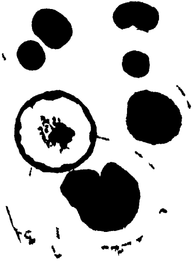

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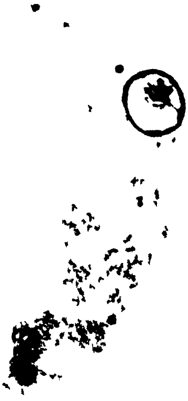

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PLATE 11

Figures 6, 7, 8, 9, 10 are camera lucida drawings of cultural material stained with iron haematoxylin. Figures 11, 12, 13 are camera lucida drawings of fresh material. × 2500.

Fig. 6. Amoeba from a L.E.A. culture of strain No. 38 to which washed human erythrocytes were added. Note the ingested erythrocyte.

Fig. 7. Amoebae from an L.E.A. culture of strain No. 42 showing an elongated rod at the posterior end.

Fig. 8. Amoeba from a 24-hour culture of strain No. 38 in L.E.A. medium. Note the ingested chain of *Streptococci*, the protoplasmic spicules, and symmetrical arrangement of the pseudopods.

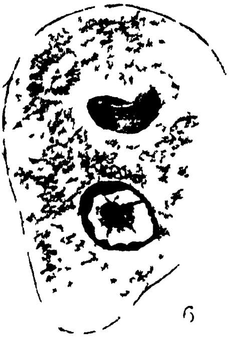
Fig. 9. Amoeba from the same culture showing 3 or 4 rather degenerate, ingested erythrocytes and the presence of numerous finger-like pseudopods extended in all directions.

Fig. 10. Amoeba from an L.E.A. culture of strain No. 33. Note the rather flattened, spread-out appearance of the organism with pseudopodia on several sides.

Fig. 11. Motile amoeba from culture No. 42, showing the broad pseudopod and the trailing protoplasmic spicules.

Fig. 12. Motile amoeba from an L.E.A. culture of strain No. 38. Note the clear pseudopod, the elongated form, and the adhesive posterior cone, surrounded by bacteria. Nucleus distinct.

Fig. 13. Motile amoeba from case 38 in a culture of L.E.A. Medium. Note the very spread-out type of pseudopodal formation all around the endoplasm, and the numerous ingested bacteria.



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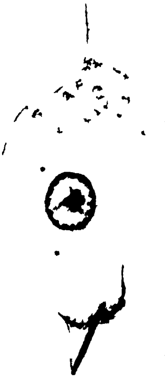

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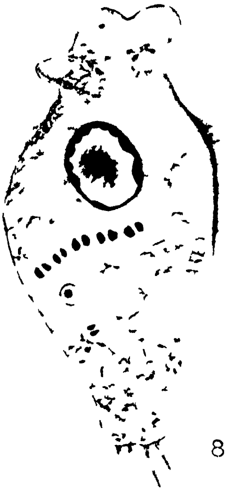

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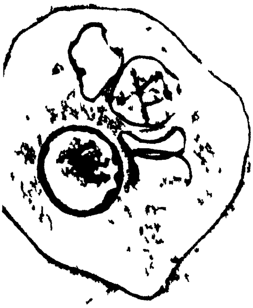

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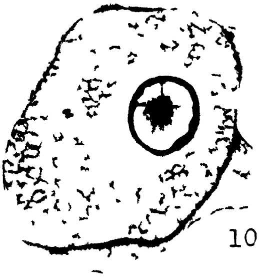

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PLATE 12

All figures are camera lucida drawings of cultural material stained with iron haematoxylin. × 2500.

Fig. 14. Amoeba from an L.A. culture of strain No. 13 without glucose. Note the large size and the granular halo around the karyosome.

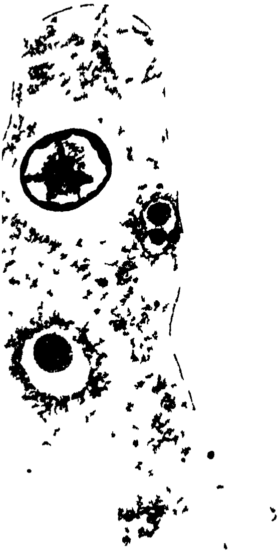
Fig. 15. Amoeba from an L.A. culture of No. 13 plus 0.5 c. c. glucose, showing the large vacuoles and tendency to pseudopod extrusion in several directions.

Fig. 16. Amoeba of strain No. 13 from the same medium. Note the large vacuoles and the broad pseudopod.

Fig. 17. Amoeba from an L.E.A. culture of No. 36. Note the elongated form, the adhesive posterior cone, and the equal distribution of ectoplasm on each side of the anterior endoplasm.

Fig. 18. Amoeba from an L.E.A. culture of No. 38 showing the faint, grayish staining spherical yolk bodies.

Fig. 19. Amoeba from the same culture of No. 38 showing many small included bodies and the tendency to extrude short finger-like pseudopods at the posterior end.



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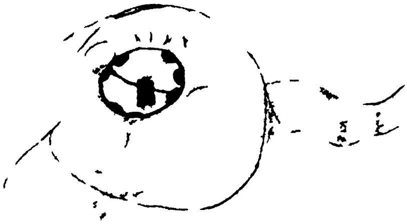

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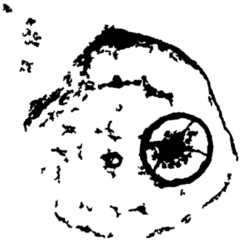

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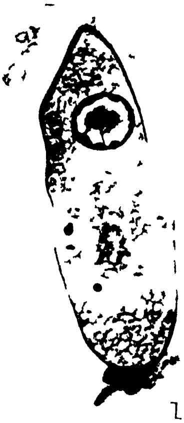

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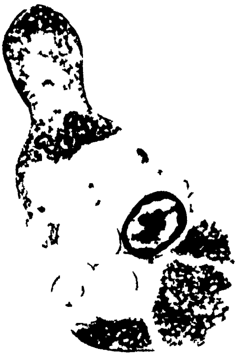

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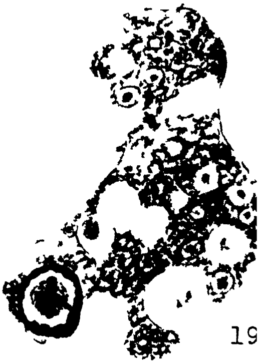

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THE BEHAVIOR OF ENDAMOEBA DYSENTERIAE IN MIXED CULTURES WITH BACTERIA

BY

CHARLES A. KOFOID AND EDNA HANNIBAL WAGENER

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CONTENTS

	PAGE
Introduction	127
Acknowledgments	127
Material and methods	128
Cases and cultures	129
Experimental	133
Summary and conclusions	148
Literature cited	149
Explanation of plates	150

INTRODUCTION

The cultivation of *Endamoeba dysenteriae* has been attempted by many investigators but probably the only successful results are those reported by Boeck (1924), who has continuously propagated this amoeba over a period of several months.

During the past year we have attempted in this laboratory to cultivate *Endamoeba dysenteriae* from acute and chronic human cases of amoebiasis, to determine if possible its optimum medium requirements and the conditions which influence encystment, and to isolate it in pure culture.

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MATERIAL AND METHODS

Following the technique reported by Boeck (1924) we have attempted during the past year to cultivate *E. dysenteriae* from fifteen cases of amoebiasis. Three of these cases showed marked symptoms of dysentery, the stools being composed chiefly of blood and mucus. These stool specimens, which contained numerous motile amoebas, were used for inoculating culture media.

The remaining eleven cases were chronic in character. Motile amoebas were obtained only after the patient had taken a saline purge. No blood or mucus was present in any of the stools from which cultures were made.

The culture medium used by us for routine work is with a few modifications identical with that used by Boeck. Four eggs which have been washed in 90 per cent alcohol for 10 to 15 minutes are broken into a sterile flask containing glass beads and the whole shaken with 50 c. c. of Locke's solution. This is tubed by means of a sterile tubing apparatus, about 4 c. c. of the medium being allowed for each tube. The tubes are then slanted in the autoclave and autoclaved 20 minutes at 15 lbs. pressure with all valves closed. This produces a firm smooth slant. Boeck (1924) covered this slant with eight parts of Locke's solution and one part of human serum, his E.L.S. medium. Later he modified this and covered the slants with Locke's solution in which the whites of two eggs were shaken. Our Locke's solution is prepared as follows:

NaCl	9. gms.
$CaCl_2$	0.2 gms.
KCl	0.4 gms.
$NaKCl_3$	0.2 gms.
Dextrose	0.25 gms.
H_2O	1000 c. c.

The whole is filtered, flasked, and autoclaved at 15 lbs. for 15 minutes. The greatest care is necessary in the preparation of the Locke's solution. If on autoclaving it becomes a brownish color or a sediment precipitates out, it is unsatisfactory, as the amoebas grow poorly, if at all. Boeck (1924) used 2.5 gms. of dextrose to a liter of Locke's solution, or ten times the amount present in our solution. We found that the smaller quantity of dextrose gave better growth of the amoebas due to difference in the amount of acid produced from the dextrose by the bacteria present in the cultures.

Except where otherwise stated we have covered the coagulated egg with 10 c. c. of Locke-albumen prepared by thoroughly shaking the whites of two eggs (which have been washed in 90 per cent alcohol) in 1000 c. c. of sterile Locke's solution. Sterile N/20 HCl is then added to bring the pH value down to 7.6 to 7.8. We refer to this as our egg-Locke-albumen medium or E.L.A. medium. This should be passed through a Berkefeld filter to insure sterility.

Initial cultures have been made by inoculating warmed tubes of medium with a loopful of mucus or fecal material from a fresh stool known in every case to contain *E. dysenteriae* in the motile phase. The culture tubes are then incubated at 37.5° C. and examined at the end of 24 and 48 hours for the presence of amoebas. Sterile Pasteur pipettes are used to transfer cultures and to remove material for microscopic examination Identification of the amoebas present in the stools and in the cultures has been made from smears fixed in hot Schaudinn's fluid and stained with haematoxylin.

The amoebas are always to be found creeping upon the surface of the coagulated egg slant. We have also learned that better growth of the amoebas results if the supernatant liquid medium which contains a heavy growth of bacteria is decanted off and replaced with fresh Locke-albumen. It has been found possible to prolong the life of a culture for nine days by replacing the Locke-albumen twice daily. Routinely subcultures have been made every 48 hours and the Locke-albumen replaced once when the cultures are approximately 24 hours old.

In our later work it was found that Locke's solution containing 0.5 per cent of whole rabbit blood gave better growth of the amoebas than did the Locke-albumen when used to cover the coagulated egg slants.

CASES AND CULTURES

Two formed specimens from chronic cases were cultured May 12, 1924. Eleven cultures were made on E.L.S. medium from each of the cases. These cultures were examined at the end of 24 and 48 hours. No amoebas were present in any of the cultures either time they were examined. A very heavy growth of both bacteria and *Blastocystis* took place. The cultures were discarded after 4 days. No further attempts were made to culture *E. dysenteriae* until September 11, 1924, when a warm specimen was obtained which contained numerous motile amoebas. The stool was formed, a few flakes of mucus adhering to the outside of the pellets. These flakes were cultured but at the end of 24 hours the cultures were negative for amoebas, although numerous *Blastocystis* and *Trichomonas hominis* were present. A fourth attempt was made on October 10, 1924. This was also a chronic case and motile amoebas were obtained only after the patient had taken magnesium sulphate. The specimen was about three and one-half hours old at the time the cultures were inoculated. Ten cultures were made. The following day a single amoeba was found in one tube which contained only a few *Blastocystis*. In the other tubes there was only a heavy growth of *Blastocystis*. The *Blastocystis* increased in amount and at the end of 48 hours both subcultures and original tubes were negative for amoebas. The fact that *Blastocystis* will grow in serum media has been demonstrated by Lynch (1922, *a, b*) and by Knowles and Das Gupta (1924).

Case 1. On October 27, 1924, we obtained a stool specimen from an acute case of amoebiasis in a patient at Letterman Hospital recently returned from the Philippines, who had been previously treated for amoebiasis. The stool specimen was liquid in character and composed wholly of blood and mucus. A direct smear examined under the microscope averaged ten amoebas to a single high power field. Stained slides showed typical *E. dysenteriae* present with ingested red blood cells (pl. 13, fig. 1). These amoebas were in some instances as much as 25 to 37 microns in diameter. Twelve tubes were inoculated immediately from this specimen, six of E.L.S. and Locke's solution and six of E.L.A. medium. A drop of fresh human blood was added to each tube before the inoculations were made. The cultures were kept warm in a thermos bottle during their transportation from Letterman Hospital to the laboratory. The remainder of the fecal specimen, which had been placed in a warm thermos bottle for transportation, was found to contain only one amoeba to a high power field of the microscope, where it had contained ten when examined two hours previously. Four more tubes of culture medium were inoculated. At the end of 24 hours the cultures were examined for growth. The four tubes inoculated after transportation of the specimen to the laboratory were negative. All twelve tubes inoculated at Letterman Hospital within 15 minutes of the time the specimen was passed showed heavy growth of a large actively motile amoeba, with hyaline pseudopodia. Many of them contained ingested red blood cells. In stained preparations these amoebas were found to have the typical nuclei of *E. dysenteriae* (pl. 13, fig. 2). No other types of amoebas were found either in the original smears or in any of the cultures. It is important to note that *not one of these cultures* made from this case (spoken of hereafter as case 1) contained any *Bldstocystis.* Both media, the E.L.S. and the E.L.A., were successful for the growth of the amoebas, but the amoebas multiplied more rapidly in the medium containing serum; they also died out more rapidly in it. No subcultures could be made from tubes more than 48 hours old. Cultures on E.L.A. reached their maximum growth at the end of 48 hours, and often contained active amoebas at the end of 4 days. No doubt this difference was due to the fact that a much heavier bacterial growth occurred when serum was present.

This case was recultured on December 22, 1924. Twelve tubes were again inoculated. Only four of these cultures were positive for amoebas at the end of 48 hours. Between October and December this patient had received local treatment which cleared up the dysenteric symptoms for some time.

CASE 2. Cultures were made from a second acute case on December 5, 1924. This patient had formerly been a soldier in the Philippines and had received at least one series of treatments for amoebiasis. The fecal specimen received at this laboratory was semisolid in character, with large flakes of blood and mucus. Both *E. dysenteriae* and *E. coli* were present in the motile phase in both fresh and stained preparations (pl. 13, fig. 3). Ten tubes of E.L.A. (pH 7.4) to which a drop of fresh human blood had been added were inoculated before the specimen was more than a half-hour old.

All of the cultures later showed the two amoebas to be present. One type with the characteristics of *E. dysenteriae* was most numerous; the other, larger, more sluggish form with granular pseudopods was identified as *E. coli.* Both *E. coli* and *E. dysenteriae* persisted in the cultures until December 15, 1924, when *E. coli* disappeared, leaving only *E. dysenteriae* (pl. 13, figs. 4, 5). This case was recultured at the physician's office. The culture medium was inoculated directly from swabs made from the ulcers of the colon during a sigmoidoscopic examination.

Again both forms of amoebas were present in the cultures. *E. coli* died out after the cultures had been through five transfers during a period of 11 days. No *Blastocystis* were present in any of the cultures.

CASE 3. December 12, 1924. This patient had lived in Manila from 1899 to 1905. There was a history of amoebic liver abscess during the period he was in Manila. The fresh stool specimens were composed largely of blood, pus, and mucus and numerous motile amoebas were present. Six cultures and four slides were made from this specimen. On December 12, 1924, two fresh stool specimens were received, both composed of blood, pus, and mucus and rich in amoebas. Four cultures were made from each of these specimens. All the cultures made on each occasion were positive for amoebas. Both *E. coli* and *E. dysenteriae* were present in stool smears and also in the cultures (pl. 14, figs. 6, 7, 8). None of the cultures contained *Blastocystis.*

Fresh warm stool specimens from six chronic cases of amoebiasis have since been cultured. In all except one case cultures were negative for amboebas at the end of 24 hours. Numerous *Blastocystis* were present each time. One of these cases which was cultured on three different occasions showed a few amoebas and numerous *Blastocystis.* The strain was carried through three or four transfers, but the *Blastocystis* became so numerous that the amoebas were destroyed.

The presence of *Blastocystis* lowers the hydrogen-ion content of the medium to pH 5.0 or less, which is apparently fatal to the amoebas.

Twenty cultures made from the pus of a liver abscess case were found to contain a hemolytic streptococcus. No amoebas were present. On another occasion cultures were made from the duodenal contents of a case which had previously showed *E. dysenteriae* present in stained smears of centrifuged duodenal contents. The cultures were negative for amoebas.

Only one attempt has been made thus far to culture from cysts. One gram of a stool specimen containing numerous cysts of *E. dysenteriae* was emulsified in 2 per cent HCl for 24 hours, the suspension being shaken frequently. At the end of 24 hours the acid was decanted and the sediment containing cysts washed several times in distilled water. Eight tubes of culture medium were inoculated with this sediment as follows:

2 tubes coagulated egg covered with Locke-albumen.
2 tubes coagulated egg covered with Locke--albumen + 1 per cent tyrosin.
2 tubes coagulated egg covered with Locke-albumen + 0.1 per cent pepsin.
2 tubes blood agar covered with Locke-albumen.

The tubes were examined at the end of 48 hours and again at the end of the fifth and seventh days. The results from these cultures are tabulated in the following table.

TABLE 1

RESULTS FROM CULTURING CYSTS OF E. DYSENTERIAE

Culture medium	Microscopic findings		
	2 days	5 days	7 days
1 coag. egg Locke-albumen	Cysts present, *Blastocystis.*	Cysts	*Blastocystis,* no cysts.
2 coag. egg Locke-albumen	Large gram pos. bacteria.	Cysts	No amoeba.
3 coag. egg Locke-albumen+ 0.1% tyrosin.	Cysts present, *Blastocystis.*	Occasional cyst	No amoeba.
4 coag. egg Locke-albumen+ 0.1% tyrosin.	Cysts present, *Blastocystis.*	Occasional cyst	No amoeba.
5 coag. egg Locke-albumen+ 0.1% pepsin.	Cysts present, *Blastocystis.*	No cysts; no amoeba, numerous blastocystis.	No amoeba.
6 coag. egg Locke-albumen+ 0.1% pepsin.	Cysts present, *Blastocystis.*		
7 blood agar Locke-albumen.	Cysts present, *Blastocystis.*		
8 blood agar Locke-albumen.	Cysts present, *Blastocystis.*		

It was found that 24 hours' treatment of feces with 2 per cent HCl was insufficient in this case to destroy the *Blastocystis.*

Although the *E. dysenteriae* cysts disappeared from every tube inoculated, we were not able to determine whether this disappearance was due to the disintegration of the cysts or to excystment, the excysted amoebas being killed by the presence of the *Blastocystis.* No motile amoebas were observed in any of the many slides examined.

EXPERIMENTAL

During the first few weeks of experimental work with the three strains of *E. dysenteriae* which we have continuously propagated in artificial media in this laboratory for several months, it was realized that accurate results could be obtained only with pure cultures. We have continuously attempted to obtain *E. dysenteriae* in pure culture, thus far without success. As growth of the amoebas can only be determined by microscopic examination, the technique followed in all examinations is as follows.

A sterile Pasteur pipette is inserted in a culture tube until the tip of the pipette touches the base of the egg slant. A sufficient quantity of the sediment and liquid is removed by means of a rubber bulb to make a good cover-glass preparation. This is placed on a slide and covered with a cover slip. The number of amoebas in the whole cover-glass preparation is then counted under the low power of the microscope. While this method offers many inaccuracies, it seemed the best adapted to our needs.

No definite conclusions can be drawn from the following tables as results in every case are modified by the heavy growth of bacteria in the cultures. This bacterial contamination consists of a *Streptococcus* of the *viridans* type, *B. coli, B. lactis aerogenes,* and a large gram positive rod which has not been identified. *B. lactis aerogenes* is especially troublesome in the cultures, as in tubes containing the raw white of egg it produces a stringy viscous mucus which makes the handling of the cultures difficult.

As we have previously stated, we found early in this work that the character of the Locke's solution used to layer on to the coagulated egg is the most important factor in the continuous propagation of *E. dysenteriae.* Frequently one unit measure of Locke's solution prepared by the routine method when used in culture media would result in many negative cultures and a scarcity of growth of amoebas in positive tubes, while another measure, prepared by the same method, would produce a heavy growth of amoebas. We therefore prepared several flasks of Locke's solution, omitting from each flask a single ingredient normally present in the solution. These solutions were autoclaved and egg albumen added to each. Tubes of coagulated egg were covered with these solutions and inoculated with 0.5 c. c. of sediment from a known positive culture. The results of these inoculations are tabulated in table 2.

It will be noted that the number of amoebas in this series of tests exceeded the controls only in the cultures from which dextrose was omitted. This difference may be explained by the fact that a heavier bacterial growth occurred in the controls which contained .025 per cent of dextrose. Series II, III, IV, and V show clearly that each ingredient is necessary for the growth of *E. dysenteriae.* Nor would *E. dysenteriae* grow on Locke-albumen without the coagulated egg slant. Howitt (1925), on the other hand, has found that if the Locke's solution is suitable the coagulated egg is not necessary for the growth of *E. gingivalis,* and Hogue (1921) has used ova-mucoid media for the cultivation of *Trichomonas hominis.*

TABLE 2

GROWTH OF AMOEBAS IN LOCKE'S SOLUTION FROM WHICH DIFFERENT INGREDIENTS OF THE SOLUTION WERE OMITTED

Solution	Number of amoebas per cover-slip preparation 24 hours	Number of amoebas per cover-slip preparation 48 hours
I		
pH 8.0		pH 7.0
NaCl ...... 0.9 gms.	30 amoebas	50 amoebas
$CaCl_2$ ...... .02 gms.		
KCl ...... .04 gms.		(fewer bacteria than in controls)
$NaHCO_3$ ...... .02 gms.		
H_2O ...... 100 c. c.		
Control, pH 7.8	17	20 pH 6.4
II		
pH 8.4		
NaCl ...... 0.9 gms.	1	0
KCl ...... .04 gms.		
$NaHCO_3$ ...... .02 gms.		
H_2O ...... 100 c. c.		
Dextrose ...... .025 gms,		
Control, pH 7.8	10	12
III		
pH 8.8		
NaCl ...... 0.9 gms.	4	3
$CaCl_2$ ...... .02 gms.		
$NaHCO_3$ ...... .02 gms.		
H_2O ...... 100 c. c.		
Dextrose ...... .025 gms.		
Control, pH 7.8	25	35
IV		
pH 8.1		
$CaCl_2$ ...... .02 gms.	0	0
KCl ...... .04 gms.		
$NaHCO_3$ ...... .02 gms.		
H_2O ...... 100 c. c.		
Dextrose ...... .025 gms.		
Control, pH 7.8	22	25
V		
pH 7.8		
$CaCl_2$ ...... .02 gms.	0	0
KCl ...... .04 gms.		
H_2O ...... 100 c. c.		
Dextrose ...... .025 gms.		
Control, pH 7.8	23	23

The question therefore arose as to whether the coagulated egg slant served merely as a support for *E. dysenteriae* to creep upon or whether the egg was ingested by the amoebas. We were also desirous of finding a simpler medium if possible. A number of kinds of media were inoculated with strains I, II, III of *E. dysenteriae,* 0.5 c. c. of sediment from positive cultures being used for inoculation. The results of strain I are recorded in the following table.

While we have recorded in this table only the growth of strain I, with but one exception strains II and III gave the same results. Strain III failed to grow in all culture media containing broth. Inasmuch as the presence of a very few amoebas in a culture might be due to the amoebas continuing to survive in the medium rather than to their multiplication, we have considered ten or more amoebas to a cover-slip preparation adequate indication of growth. On this basis growth occurred only in those cultures which contained either coagulated egg or blood agar. The bacterial growth was far heavier on the blood agar media and the growth of the amoebas correspondingly lower. The addition of 0.5 per cent cystin, yeast extract, or sterile raw potato did not appear to influence growth of the amoebas when coagulated egg was present, nor did any of these substances serve as a substitute for coagulated egg or blood, as has been found with cystin in the cultivation of *B. tularense* (Francis, 1923) and with raw potato and yeast extract in the cultivation of *B. influenzae* (Thjotta and Avery, 1921 *a*, *b*). We judged from these results that *E. dysenteriae* required either the coagulated egg or the blood in blood agar slants for at least a part of its food supply. In 24-hour cultures on Locke-albumen coagulated egg, *E. dysenteriae* frequently contains numerous minute glistening bodies. In fresh material there was some question as to whether they were food granules or ingested bacteria. Smears fixed in methyl alcohol and stained by Gram's method showed the amoebas as faintly staining gram-negative masses with no ingested bacteria. On one occasion only have we found bacteria ingested by amoeba in 24-hour cultures (pl. 14, fig. 8). However, 72-hour cultures gave different results. In fresh preparations the amoebas are rounded and appear as highly phagocytized leucocytes. The same smear stained by Gram's method showed the cytoplasm of the amoeba completely filled with both gram-negative and gram-positive bacteria. In haemotoxylin-stained smears of these old cultures there is marked evidence of degeneration of the nucleus (pl. 14, figs. 11, 12, 13, 14, 15).

TABLE 3

GROWTH OF E. DYSENTERIAE ON DIFFERENT CULTURE MEDIA (STRAIN I)

Culture media used	Original pH	Number of amoebas per cover-slip preparation after 48 hours	Final pH
1. Infusion peptone broth	7.0	0	6.3
2. Infusion peptone broth on coagulated egg	7.0	1	6.2
3. 15% infusion peptone broth in Locke's solution	7.2	0	6.2
4. 15% infusion peptone broth in Locke's solution on coagulated egg	7.2	5	6.2
5. 15% infusion peptone broth in Locke's solution plus 0.25% dextrose	7.0	0	5.0
6. 15% infusion peptone broth in Locke's solution plus 0.25% dextrose on coagulated egg	7.0	1	5.6
7. Locke's solution	7.4	1	
8. Locke's solution on coagulated egg	7.4	10	6.0
9. Locke's solution with slice of sterile raw potato	7.5	0	
10. Locke's solution plus .05% cystin plus sterile raw potato	7.5	0	
11. Locke-albumen plus sterile raw potato	7.4	1	
12. E.L.A. plus sterile raw potato	8.2	30	
13. Infusion peptone broth, coagulated egg, .05% cystin	7.0	20	
14. Locke's solution plus .05% cystin	7.2	0	
15. Locke's solution, coagulated egg plus .05% cystin	7.0	20	
16. Locke-albumen plus 10% yeast extract	7.0	0	
17. E.L.A. plus 10% yeast extract	7.0	9	
18. Locke-albumen plus 1% yeast extract	7.0	10	
19. E.L.A. plus 1% yeast extract	7.0	18	
20. Heated Locke-albumen	7.4	0	
21. Heated Locke-albumen plus 1% haemoglobin	7.4	0	
22. Peptic digest agar slant with Locke-albumen	7.6	0	
23. 1% agar slant, with Locke-albumen	7.6	0	
24. Infusion agar slant with Locke-albumen	7.6	0	
25. Blood agar slant with Locke-albumen	7.6	12 (heavy growth of bacteria)	5.0
26. N.N.N. slant with Locke-albumen	7.6	11 (heavy growth of bacteria)	5.0
27. Brain medium (aerobic)	7.2	0	
28. Brain medium (anaerobic)	7.2	0	

Fresh preparations from 24- and 48-hour cultures when stained by Sudan III show numerous small bodies which take up the stain. These appear to correspond in shape and size with the glistening bodies observed in fresh preparations. Sudan III was also taken up by the fat globules in the medium. It is therefore possible that *E. dysenteriae* ingests lipoidal substances from the egg yolk or blood serum, and that it is this material which appears as glistening bodies and which stains with Sudan III. To determine if *E. dysenteriae* is able to ingest other fats, cod liver oil was added to white of egg slants. This medium was prepared by adding to egg albumen 1 per cent cod liver oil in which had been dissolved 10 per cent Sudan III. The whole was thoroughly shaken in a sterile flask containing beads until an emulsion of a strawberry pink color was obtained. This was tubed with sterile precautions, slanted autoclaved 20 minutes with all valves closed.

This medium was then covered with 10 c. c. of Locke-albumen. In order to control the experiment, 0.5 c. c. of sediment from a known positive tube was inoculated into, respectively, a control and tube of coagulated albumen and Sudan III. The findings are given in table 4.

TABLE 4

EFFECTS OF CULTURING E. DYSENTERIAE ON COAGULATED WHITE OF EGG WITH SUDAN III IN COD LIVER OIL

Culture media used	Number of amoebas in cover-slip preparation at end of 24 hours	Number of amoebas in cover-slip preparation at end of 48 hours
1. Coagulated white of egg, cod liver oil and 1% Sudan III.	35, fine red granules in cytoplasm.	15, small rounded granules.
2. E.L.A. control	20, no granules	10, no granules.
3. Coagulated white of egg, cod liver oil and 1% Sudan III.	30, red granules	0
4. E.L.A. control	15, no granules	7, no granules.
5. Coagulated white of egg, cod liver oil and 1% Sudan III.	50, red granules	4, rounded appeared dead.
6. E.L.A. control	20, no granules	17, no granules.

It will be noted that excellent growth occurred on this medium by the end of 24 hours. In direct smears, when examined under the microscope, more than 50 per cent of the amoebas were found to contain small red granules. No such granules were visible in the amoebas from the control tubes which contained no cod liver oil and no Sudan III. This would suggest that *E. dysenteriae* ingests oils in the form of minute droplets. Howitt (1925), conducting similar experiments with *Endamoeba gingivalis,* found that large droplets of fats were ingested by that *Endamoeba.* No subcultures could be obtained from the tubes containing Sudan III.

Later a series of experiments were made to determine if cod liver oil, when added to E.L.A. media, would increase the growth of the amoebas, or if it could be substituted for coagulated egg. When cod liver oil was added to E.L.A. medium, it immediately separated and floated to the surface. To control this medium, sterile paraffin oil was added to E.L.A. tubes. To other tubes containing infusion agar, 2 per cent cod liver oil was added. The tubes were shaken as they cooled, then covered with Locke-albumen. The findings at the end of 48 hours are recorded in table 5.

TABLE 5

THE GROWTH OF E. DYSENTERIAE IN MEDIA CONTAINING OILS

Media used	Original pH	Number of amoebas in cover-slip preparation end of 48 hours	Final pH
E.L.A.+1% cod liver oil	8.0	75	6.8
E.L.A.+.1% cod liver oil	8.0	35	6.8
E.L.A.+.01% cod liver oil	8.0	30	6.7
E.L.+1% cod liver oil	7.4	100	6.6
E.L.+.1% cod liver oil	7.4	50	6.4
E.L.+.01% cod liver oil	7.4	30	6.8
E. L. control	7.4	2	6.4
E.L.A. control	8.0	20	6.6
1. Infusion agar slant L.A.+2% cod liver oil	8.0	3	6.8
2. Infusion agar slant L.A.+2% cod liver oil	8.0	7	6.6
3. Infusion agar slant Locke's+2% cod liver oil	7.4	3	6.9
1. E.L.A.+5% liquid paraffin	8.0	140	6.8
2. E.L.A.+5% liquid paraffin	8.0	102	6.8
3. E.L.A.+5% liquid paraffin	8.0	127	6.7
4. E.L.A.+5% liquid paraffin	8.0	135	6.8
1. E.L.A. control (aerobic)	8.0	50	6.4
2. E.L.A. control (aerobic)	8.0	72	6.3
3. E.L.A. control (aerobic)	8.0	81	6.4
4. E.L.A. control (aerobic)	8.0	43	6.2

Growth of *E. dysenteriae* on agar slants containing 2 per cent cod liver oil was unsatisfactory. The excellent growth which occurred in all tubes containing oil as compared with the aerobic E.L.A. controls would indicate that the partial anaerobic conditions produced affected the growth of the amoebas far more than the kind of oil added to the culture. Again we are unable to state whether the marked increase in growth of the amoebas in these anaerobic cultures was due to their preference for anaerobic conditions for multiplication or whether the decreased growth of the bacteria in these cultures made the growth conditions more favorable for the amoebas. It will be necessary to repeat these experiments with pure cultures of *E. dysenteriae* to determine this point.

It was apparent that the hydrogen-ion content of the medium affected the growth of *E. dysenteriae,* as E.L.A. medium when unadjusted had usually a pH of 8.2 or 8.4 after standing a short time. Culture medium adjusted to pH 7.8 when inoculated with 0.5 c. c. of a 48-hour culture dropped upon inoculation to pH 7.2. When tested at the end of 24 hours' incubation, it had dropped to pH 5.2 to 5.0, after which it again rose to pH 6.2 to 6.4 at the end of 48 hours. Inoculations were therefore made into tubes of culture media whose hydrogen-ion content had been adjusted at varying points. Observations were then made in regard to the growth of *E. dysenteriae* and the pH at the end of 24 and 48 hours. The results are tabulated below:

TABLE 6

EFFECTS OF THE HYDROGEN-ION CONTENT OF THE MEDIUM ON THE GROWTH OF E. DYSENTERIAE (STRAIN I)

Initial pH when inoculated with 0.5 c. c. culture	Number of amoebas in a cover-slip preparation at the end of 24 hours	pH at end of 24 hours	Number of amoebas in a cover-slip preparation at the end of 48 hours	pH at end of 48 hours
5.8	2	below range	5	6.0
6.4	1	5.0	4	6.2
7.2	12	5.2	7	5.9
8.0	2	5.1	23	6.2
8.2	3	5.4	5	6.4
8.4	10	5.3	10	6.6
control 7.8	10	5.1	20	6.4

All three strains of *E. dysenteriae* were run in duplicate. Each strain showed the maximum growth in media of the initial hydrogen-ion content of 7.8 to 8.0 with a final pH of 6.2 to 6.4.

Boeck (1924) made his initial cultures of *Endamoeba* in E.L.S. medium which contained a drop of fresh human blood, the addition of the blood apparently stimulating the multiplication of the amoebas. We also found that the addition of human blood to E.L.A. markedly increased the growth of the amoebas especially during the first 24 hours of their growth; after that time the increased growth of the bacteria seemed to inhibit or decrease further growth of the amoebas. Inasmuch as *E. dysenteriae* multiplied very rapidly during the first 24 hours on blood agar slants prepared with rabbit blood, the question arose as to whether or not the blood of other mammals could be substituted for human blood. One per cent whole blood from the following animals, cat, rat, guinea pig, and rabbit, respectively, was therefore added to E.L.A. media. Human blood in 1 per cent amounts served as a control. Very heavy growth of the amoebas took place in all tubes but the number of red blood cells present frequently obscured the amoebas. The experiment was therefore repeated using 0.5 per cent whole blood and 0.5 per cent haemolized whole blood in duplicate tests. With this amount of blood the bacteria did not multiply quite so rapidly and the growth of the amoebas seemed equally as good. There was apparently no difference in the rate of growth between tubes containing laked and unlaked blood. In repeated experiments the poorest growth always occurred in cultures containing rat blood. This may have been purely accidental, in that each tube may have received fewer amoebas in its initial inoculation. On the other hand, Kessel's (1923) finding that the percentage of infections with *E. dysenteriae* among amoeba-free rats is higher than among rats already infected with rat amoebas introduced another possibility; namely, that rat blood may contain certain antibodies which affect to a slight extent the growth of *E. dysenteriae* in culture. This also seems plausible in the light of the findings of one of us (Wagener, 1924), that the blood of cats which had had infections with *E. dysenteriae* of more than a week's duration gave a positive precipitin test with antigens prepared from scrapings from ulcers of the intestine of cats. The rat blood added to these cultures was obtained from adult rats of the stock supply. All these rats were infected with rat amoebas.

Other than the difference noted with rat blood there seemed no difference of growth in tubes which contained cat, guinea pig, rabbit, or human blood, respectively, and amoebas were found ingesting red blood cells in all tubes. In all culture media containing blood there was an increased growth of bacteria. The viscous mucus always present in media containing albumen increased in amount and made the handling of these cultures especially difficult. It was therefore important to find a medium which did not contain raw egg albumen. Varying amounts of whole and laked blood were added to Locke's solution and used to cover the egg slants without the egg albumen being added. The bacteria produced little or no mucus on this medium while the growth of the amoebas was very good. There was also the additional advantage that sterile rabbit blood could always be obtained and added in 0.5 per cent amounts to sterile Locke's solution with little difficulty and without having to adjust the pH value. Locke-albumen, on the contrary, was frequently contaminated, and it was always necessary to pass it through a Berkefeld filter to insure its sterility, a process involving a great deal of time when large quantities of media were necessary. Moreover, the pH value of Locke-albumen had always to be adjusted with N/20 HCl, and it does not remain constant due to autolysis of the egg albumen. For these reasons E.L.B. medium (coagulated egg and Locke's solution containing 0.5 per cent whole rabbit blood) has since been used for the carrying of stock cultures or for experimental work save when special medium was required. A series of experiments were then made to determine the effects on the growth of *E. dysenteriae* of haemoglobin alone as compared to whole blood and blood serum. Coagulated egg slants were covered with Locke's solution to which was added laked, washed red blood cells, serum, or whole blood in sufficient quantity to make a 0.5 per cent dilution. The results are tabulated below. Rabbit blood was used in these experiments.

TABLE 7

COMPARATIVE GROWTH OF E. DYSENTERIAE ON MEDIA CONTAINING SERUM, HAEMOGLOBIN, OR WHOLE BLOOD

Medium	Number of amoebas in cover-slip preparation at end of 24 hours	Number of amoebas in cover-slip preparation at end of 48 hours
E.L.+0.5% laked washed RBC	0	25
E.L.+0.5% laked washed RBC	10	35
E.L.+0.5% laked whole blood	30	30
E.L.+0.5% laked whole blood	20	10
E.L.+0.5% serum	75	15
E.L.+0.5% serum	50	24
E.L.+0.5% whole blood	100	28
E.L.+0.5% whole blood	75	22
E.L.A. control	3	3
E.L.A. control	35	20

It will be observed that the greatest number of amoebas occurred in media containing 0.5 per cent of whole blood, while that prepared with laked, washed red blood cells showed the least growth. We would judge from this that serum is a necessary growth-producing factor in the medium. In table 2 we found that coagulated egg with Locke's solution alone will show a moderate growth of *E. dysenteriae* at the end of 48 hours. This has been found true for one or two transfers, after which the amoebas die out.

Cropper and Drew (1914) in cultivating a saprophytic amoeba found that the presence of serum in the media acted as a stimulant for excystment. Tyrosin in 0.1 per cent amounts was found by them to have the same effect and to cause, in larger quantities, encystment of the free forms. The addition of 0.2 per cent tyrosin to our cultures in E.L.B. medium did not affect *E. dysenteriae* in any way. Certainly its addition did not cause them to encyst.

In considering the encystment of *E. dysenteriae* in the human bowel it seemed possible that the presence of bile might be an important factor. Through the courtesy of Dr. L. M. Boyers of Berkeley, we were able to secure specimens of human bile for experimental purposes. Varying dilutions of bile were added to E.L.A. medium, which was then added to positive cultures. These tubes were incubated and examined at the end of 24 and 48 hours for growth of amoebas and for the presence of cysts. As the bile had been obtained by giving the patient magnesium sulphate (to open the sphincter muscle of the bile duct), controls were made by adding the same dilutions of a saturated solution of magnesium sulphate to E.L.A. medium. We also obtained cat bile without the use of magnesium sulphate by etherizing the cat and immediately removing its gall bladder. Duplicate tests were run with all three strains of *E. dysenteriae* in the case of the human bile and magnesium sulphate. Because of the small quantity of cat bile, duplicate tests could not be made with it.

Besides these cultures, equal parts of undiluted bile and culture material containing active amoebas were mixed on a slide, sealed with vaseline, and observed under the microscope. In each case the amoebas exploded in the presence of bile on the slide in from 20 to 30 minutes. It will be noted from table 8 that 1–10 dilutions of bile are sufficient to destroy active *E. dysenteriae* from cultures. As compared to the controls it appears that even higher dilutions slightly inhibit growth. Magnesium sulphate appears to have no effect on the growth curve. No cysts were found in any of the smears examined, either in fresh or stained preparations.

We can definitely state that *in vitro* the presence of bile in the dilutions used does not cause encystment during the 48-hour period in which cultures are viable.

TABLE 8

EFFECTS OF BILE ON E. DYSENTERIAE IN CULTURES OF COAGULATED EGG LOCKE-ALBUMEN

Amount of bile added to culture tubes	Results 24 hours Strains			Results 48 hours Strains		
	I	II	III	I	II	III
Human bile:						
1–10 diluted in Locke-albumen	0	0	0	0	0	0
1–30	1	10	0	2	7	7
1–60	1	10	0	0	4	0
1–100	7	12	0	2	2	7
1–200	5	1	6	2	5	4
E.L.A. control	10	15	10	15	7	9
Cat bile:						
Undiluted	0			0		
1–10 diluted in Locke-albumen	0			0		
1–30	3			2		
1–60	3			3		
1–100	15			9		
E.L.A. control	7			12		
Effects of magnesium sulphate on *E. dysenteriae:*						
1–10 diluted in Locke-albumen	3	4	10	10	0	10
1–30	10	0	3	3	2	7
1–60	15	7	4	7	3	2
1–100	17	10	4	5	3	3
1–200	13	6	2	4	5	3
E.L.A. control	12	10	10	15	7	8

In connection with the work with bile, cholesterol was also added to cultures. A 10 per cent solution was made by dissolving 0.5 grams of cholesterol in 5 c. c. of ether. This was added to E.L.A. in varying amounts. As the cholesterol is insoluble in any substance but ether and hot alcohol, the cholesterol present in the ether solution immediately recrystalized and rose to the surface of the tube, making a complete seal over the top of the medium. For this reason both anaerobic and aerobic controls were run. It is our belief that the excellent growth obtained in these cultures is due to the anaerobic conditions produced and not to the presence of the cholesterol, which remained on the surface of the media. The results of these tests are shown in table 9.

TABLE 9

GROWTH OF E. DYSENTERIAE IN MEDIA CONTAINING VARYING AMOUNTS OF CHOLESTEROL

Dilutions of ether solution of cholesterol in E L A	Number of amoebas per cover-slip preparation at end of 24 hours	Number of amoebas per cover-slip preparation at end of 48 hours
0 01%	500 large, active	21
0 02%	25 large, active	43
0 03%	250 large, active	400 small rounded forms.
0 04%	275 large, active	45
0 05%	200 large, active	50 very small, rounded.
0 1%	6 large	6 large.
Aerobic control	4 small	30
Anaerobic control	750 large, active	97 small

It is noted that although heavy growth of amoebas occurred in the tubes containing cholesterol, the anaerobic control showed the greatest growth.

In a few experiments made with human gastric contents it was found that, when equal parts of gastric contents having a free hydrochloric of 34 and a total acidity of 65 were mixed with culture material on a slide and examined under the microscope, the amoebas became moribund, then exploded in from two and a half to four minutes. No attempts were made to carry out dilutions with gastric contents.

It has been stated in another part of this paper that the necessity of working with pure cultures of *E. dysenteriae* was fully realized early in the work. During the course of this problem attempts have been made to attain pure cultures. The first attempts made were to pick out single amoeba by means of the Barber technique. This failed completely, primarily because it was impossible to dilute the culture to a point where amoebas were still present in the drop while the bacteria were sufficiently diluted to get an amoeba out of the seething mass of motile organisms. Secondly, it failed because motile amoebas adhere very closely to any solid material, such as the cover slip, or, if picked up in the pipette, they adhere to the pipette and cannot be discharged. Amoebas also tend to pick up and cling to bacteria by means of the adhesive cone on their posterior ends.

Attempts were also made to obtain a pure line by the method used by Hogue (1921) in obtaining a pure line of *Trichomonas hominis.* Drops of the culture were diluted on pieces of sterile cover slips until a drop contained only one amoeba. Cover slip and all were then dropped into a tube of culture medium. In only one tube from some thirty so inoculated was it possible to find an amoeba present after 48 hours' incubation. From one tube a single amoeba was found in a cover-slip preparation but subcultures from this and the remaining tubes were negative, since the single amoeba proved to be outgrown by the bacteria.

Various dyes have also been used to inhibit the growth of bacteria in these cultures. Methylene blue, fuchsin, and acriflavine have been tried. Two methods have been used; (1) the addition of varying dilutions of the dyes to tubes of E.L.B., and (2) the treatment of sediment from positive cultures in Locke's solution containing varying dilutions of dyes for a period of several hours. The second method served better with fuchsin and methylene blue as both of these dyes lose their bactericidal action in the presence of albumens. Acriflavine, however, has been shown by Gay and Beckwith (1922) to be equally bactericidal in the presence of serum and albumens.

Fuchsin and methylene blue had no effect either upon the amoebas or upon the bacteria when added to E.L.A. in dilutions of 1:100,000, 1:10,000, and 1:1000 amounts. These two dyes when added to Locke's solution in the same dilutions and left in contact with sediment containing active amoebas still failed to inhibit the growth of the bacteria when subcultures were made to E.L.A. As will be observed from table 10, fuchsin in 1:1000 dilution slightly inhibited the growth of bacteria. As both of these dyes are poor bactericidal agents, work with them was discontinued and acriflavine used instead.

The acriflavine was added to E.L.B. in dilutions of 1:5000, 1:10,000, 1:50,000, and 1:100,000, and the cultures examined at the end of 24 and 48 hours for growth of amoebas and bacteria. As will be observed from table 11 growth of *E. dysenteriae* occurred in all dilutions of the dye. Positive subcultures, however, could not be obtained from tubes containing acriflavine at a 1:5000 dilution. As there was still marked inhibition of the bacterial flora at 1:10,000 dilution, subcultures were made into other media containing the same dilution of the dye. It was hoped that by transferring to fresh E.L.B. containing 1:10,000 dilution of acriflavine the bacteria would be further reduced and it would be possible to isolate the amoeba in pure culture. But this hope was not realized. The bacteria were not reduced below a certain point, and after eight to ten transfers in acriflavine the amoeba failed to grow when subcultured.

Strains I and II were accidentally lost after being carried through 62 and 55 transfers respectively. At the time of writing strain III has received 80 transfers.

The evidence that these strains which have been propagated on artificial medium are *E. dysenteriae* is based on their morphology.

TABLE 10

EFFECT OF METHYLENE BLUE AND FUCHSIN ON E. DYSENTERIAE AND THE BACTERIA WITH WHICH IT IS ASSOCIATED

Dilution of dye in Locke's solution	Length of time in contact: hours	Growth of amoeba in subcultures from dye solution 48 hours	Inhibition of bacteria in subcultures from dye solution 48 hours
Fuchsin:			
1–1000 dilution	3	Positive	None.
1–10,000 dilution	3	Positive	None.
1–100,000 dilution	3	Positive	None.
1–1000 dilution	6	Positive	Slight.
1–10,000 dilution	6	Positive	None.
1–100,000 dilution	6	Positive	None.
Methylene blue (Merk):			
1–1000 dilution	3	Negative	None.
1–10,000 dilution	3	Positive	None
1–100,000 dilution	3	Positive	None
1–1000 dilution	6	Negative	None.
1–10,000 dilution	6	Positive	None.
1–100,000 dilution	6	Positive	None.

TABLE 11

EFFECTS OF ACRIFLAVINE ADDED TO CULTURES ON E.L.B.

Dilution of acriflavine in culture media	Number of amoebas present in cover-slip preparation at end of 24 hours	Number of amoebas present in cover-slip preparation at end of 48 hours
1–5,000	50 amoebas—bacterial growth inhibited	30*
1–5,000	20 amoebas—some inhibition of bacteria	10*
1–10,000	75 amoebas—slight inhibition of bacteria	30*
1–10,000	90 amoebas—slight inhibition of bacteria	20*
1–50,000	100 amoebas—slight inhibition of bacteria	17*
1–50,000	70 amoebas—slight inhibition of bacteria	10*
1–100,000	50 amoebas—bacteria not inhibited over control.	6*
1–100,000	30 amoebas—bacteria not inhibited over control.	10*
Control (without dye)	20 amoebas—heavy growth of bacteria	14*

* Bacterial growth had increased.

This amoeba in cultures is actively motile, with clear, hyaline pseudopods. We have observed the ingestion of red blood cells by all three strains in cultures. In carefully stained preparations the central karyosome is compact and the peripheral chromatin is scattered over the nuclear membrane uniformly in plaques or bead-like granules. In the majority of amoebas studied in stained preparations, spoke radiations connecting the central karyosomes with the nuclear membrane are easily discernible. At mitosis the nuclear membrane remains intact. The karyosomes are connected with an intradesmose. In one dividing amoeba four chromatin granules (chromosomes) were observed and in another six large chromatin masses were lined up on the nuclear membrane.

In old cultures the nuclear membrane appears to thicken, the central karyosome disappears, and the nucleus becomes distorted and may eventually, in 96-hour cultures, disappear altogether.

SUMMARY AND CONCLUSIONS

1. *Endamoeba dysenteriae* has been grown by us in artificial media on repeated occasions from three acute cases of human amoebiasis. The stools of none of these three patients contained *Blastocystis.*

2. Cultures made from the stools of nine different individuals with chronic amoebiasis and a heavy infection with *Blastocystis* failed to give positive results, apparently because of over-growth by this organism.

3. For the growth *in vitro* of *E. dysenteriae* either egg or blood is a necessary medium requireemnt.

4. *E. dysenteriae* ingests lipoidal substances present in coagulated egg as is shown when these are stained with Sudan III.

5. *E. dysenteriae* grows better under partial anaerobic conditions than under aerobic conditions.

6. The presence of blood stimulates more rapid multiplication of *E. dysenteriae* in culture media. Rabbit, guinea pig, rat, and cat blood may be substituted for human blood.

7. Bile does not cause encystment *in vitro.*

8. No method was found for the isolation of *E. dysenteriae* in pure culture.

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EXPLANATION OF PLATES

All figures are camera lucida drawings of *Endamoeba dysenteriae* (Councilman and Lafleur) Craig from smears from human stools or cultures fixed with hot Schaudinn's fluid and stained with iron haematoxylin × 2500.

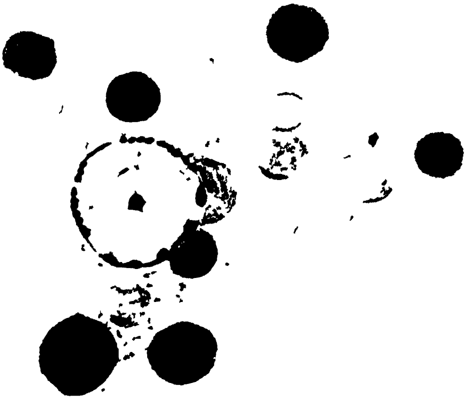
PLATE 13

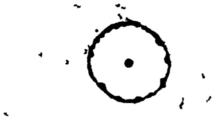
Fig. 1. Motile phase in direct smear from stool of case 1. The cytoplasm contains numerous ingested red blood cells. 35 × 27μ in diameter.

Fig. 2. Motile phase from culture of strain I after twelve transfers on E.L.A. medium. The cytoplasm is slightly vacuolated. 12μ in diameter.

Fig. 3. Motile phase in fecal smear from case 2. Three ingested red blood cells are present in the cytoplasm. Karyosome of the type frequently found in tissue. 20 × 24μ in diameter.

Fig. 4. Mitosis from 24-hour culture of strain II in E.L.A. medium. The karyosome centrosomes have divided with the intradesmose connecting. Four chromatin granules (chromosomes) are visible. The cytoplasm is highly vacuolated. 11.5μ in diameter.





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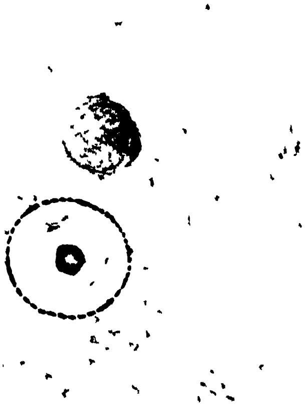

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PLATE 14

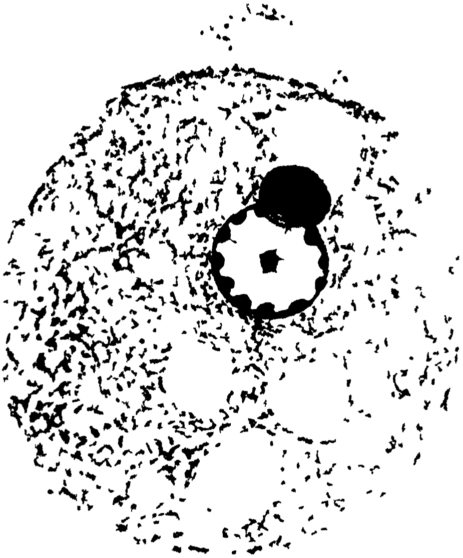
Fig. 5. Typical *E. dysenteriae* in 48-hour culture on E.L.A. medium. Note the reduction in size of the amoeba in culture as compared to the size in direct smears. Eleventh transfer. 11μ in diameter.

Fig. 6. Motile phase with ingested red blood cells in direct fecal smear from case 3. 27 × 23.5μ in diameter.

Fig. 7. *E. dysenteriae* in 24-hour culture inoculated with mucus from case 3 (first subculture). 11 × 15μ in diameter.

Fig. 8. *E. dysenteriae* in the twelfth transfer in E.L.A. medium. Twenty-four-hour culture showing numerous ingested bacteria. 20 × 24μ in diameter.

Fig. 9. Early prophase of *E. dysenteriae* (strain III) in the thirty-third transfer. Twenty-four-hour old culture. 11μ in diameter.



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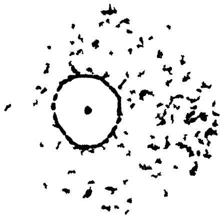

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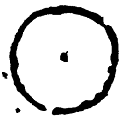

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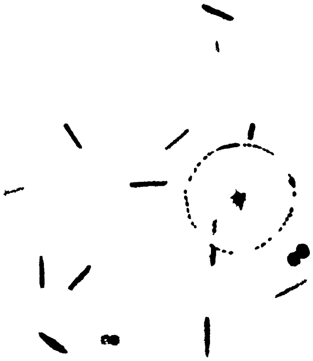
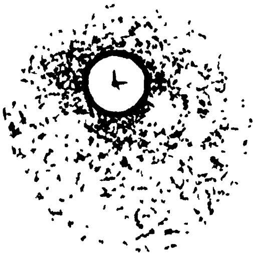


PLATE 15

Fig. 10. *E. dysenteriae* in a 24-hour culture (thirty-second transfer) of strain III. Cytoplasm very dense. 11μ in diameter.

Figs. 11, 12, 13, 14, and 15. Stages in degeneration of the nucleus in amoebas in 72-hour cultures in E.L.A. medium.



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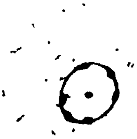

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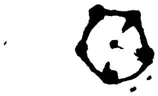

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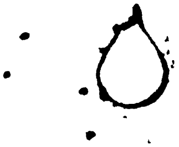

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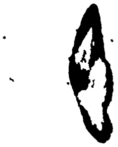

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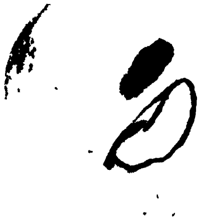

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STUDIES ON THE EFFECTS OF CERTAIN DRUGS UPON ENDAMOEBA DYSENTERIAE IN VITRO

BY

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CONTENTS

	PAGE
Introduction	155
Acknowledgments	155
Material and methods	156
Experimental effects of drugs	157
Summary and conclusions	165
Literature cited	166

INTRODUCTION

The effects of various drugs on species of the *limax* type of amoeba have been studied by various investigators in the hope of throwing light upon the treatment of amoebiasis. Sellards and Leiva (1923) have reviewed the literature upon this subject in connection with their work on the treatment of experimental amoebiasis in cats. In 1925 Boeck and Drbohlav reported continuous propagation of *Endamoeba dysenteriae* in artificial medium and their results have since been verified by Drbohlav (1925) in Europe and in this laboratory by Kofoid and Wagener (1925). In continuation of this previous work we have tested the amoebicidal action of certain drugs *in vitro* on one of our strains of *E. dysenteriae*, the causative organism of human amoebiasis. The drugs used have been those recommended in the treatment of amoebiasis. Obviously the results obtained cannot be applied directly to conditions *in vivo* in man but a comparison can be drawn on the relative effects of these drugs on this amoeba *in vitro*.

ACKNOWLEDGMENTS

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MATERIAL AND METHODS

The culture of *Endamoeba dysenteriae* used for the following experiments was obtained from an acute case of amoebiasis, the details of which have been reported in our previous paper (Kofoid and Wagener, 1925). At the time this work was started strain III had been under artificial cultivation for five months and had received seventy-eight transfers at 48-hour intervals. All cultures have been made upon coagulated egg slants covered with 10 c. c. of Locke's solution containing 0.5 per cent of defibrinated rabbit's blood (our E.L.B. medium).

Ten per cent solutions of the various drugs in Locke's were added to the culture medium in a wide range of dilutions. After addition of the drug to the culture medium, the tubes were warmed in the incubator and inoculated from 48-hour cultures known to contain *E. dysenteriae.* For every tube containing a dilution of the drug inoculated, a control tube of corresponding number was also inoculated at the same time from the same positive culture. All cultures were then incubated at 37° C. and examined for a growth at the end of 24 and 48 hours. Examination was made by removing a small quantity of sediment from the base of the egg slant by means of a Pasteur pipette. The drop thus obtained was placed on a warm slide and covered with a cover slip. The number of amoebas in this cover-slip preparation were then counted under the microscope. The results are recorded in the following tables.

TABLE 1

THE EFFECTS OF EMETIN HYDROCHLORIDE ON E. DYSENTERIAE IN VITRO

No. of culture tube	Dilution of drug in medium	Number of amoebas present in cover-slip preparation		No. of control tube	Number of amoebas present in cover-slip preparation	
		After 24 hours	After 48 hours		After 24 hours	After 48 hours
1	1–100,000	10	2	1	25	3
2	1–50,000	100	1	2	30	10
3	1–33,300	6	2	3	5	30
4	1–25,000	10	0	4	5	10
5	1–20,000	8	2	5	33	7
6	1–16,600	8	1 (dead)	6	15	5
7	1–14,200	0	0	7	30	7
8	1–12,500	1	0	8	25	6
9	1–11,100	0	0	9	12	11
10	1–10,000	1	0	10	15	3
11	1–5,000	0	0	11	10	7
12	1–3,330	0	0	12	30	5
13	1–2,500	0	0	13	17	5
14	1–2,000	0	0	14	7	4
15	1–1,660	0	0	15	25	2
16	1–1,420	0	0	16	15	3
17	1–1,250	0	0	17	4	2
18	1–1,110	0	0	18	4	3
19	1–1,000	0	0	19	15	8
20	1–200	0	0	20	25	10

No visible reduction in bacterial content of tubes containing drug as compared with controls.

TABLE 2

The Effects of Acetylarsan on E. dysenteriae in vitro

No of culture tube	Dilution of drug in medium	Number of amoebas present in cover-slip preparation		No of control tube	Number of amoebas present in cover-slip preparation	
		After 24 hours	After 48 hours		After 24 hours	After 48 hours
1	1–100,000	30	5	1	55	5
2	1–50,000	6	7	2	10	3
3	1–33,300	20	2	3	37	8
4	1–25,000	10	1	4	14	7
5	1–20,000	6	0	5	10	6
6	1–16,600	4	0	6	20	10
7	1–14,200	0	0	7	30	8
8	1–12,500	0	0	8	10	9
9	1–11,100	0	0	9	50	4
10	1–10,000	1 (dead)	0	10	10	20
11	1–5,000	0	0	11	40	5
12	1–3,330	0	0	12	10	7
13	1–2,500	0	0	13	3	10
14	1–2,000	0	0	14	10	8
15	1–1,660	0	0	15	15	6
16	1–1,420	0	0	16	3	3
17	1–1,250	0	0	17	10	6
18	1–1,110	0	0	18	10	8
19	1–1,000	0	0	19	90	10
20	1–500	0	0	20	33	10
21	1–330	0	0	21	15	7
22	1–250	0	0	22	19	3
23	1–200	0	0	23	27	13
24	1–100	0	0	24	3	5
25	1–50	0	0	25	4	6

No visible reduction in bacterial content of tubes containing drug as compared with controls.

TABLE 3

THE EFFECTS OF STOVARSOL ON E. DYSENTERIAE IN VITRO

No. of culture tube	Dilution of drug in medium	Number of amoebas present in cover-slip preparation		No. of control tube	Number of amoebas present in cover-slip preparation	
		After 24 hours	After 48 hours		After 24 hours	After 48 hours
1	1–666,000	14	2	1	25	2
2	1–333,000	7	0	2	15	2
3	1–222,000	8	0	3	100	3
4	1–166,500	10 (large)	0	4	100	2
5	1–133,200	5	0	5	75	10
6	1–111,000	7	0	6	25	3
7	1–95,112	2	0	7	Not inoculated	
8	1–83,000	0	0	8	10	10
9	1–74,000	0	0	9	2	2
10	1–66,600	0	0	10	8	8
11	1–33,300	0	0	11	10	10
12	1–22,200	0	0	12	10	10
13	1–13,320	0	0	13	30	4
14	1–11,100	0	0	14	10	11
15	1–9,514	0	0	15	10	3
16	1–8,300	0	0	16	20	6
17	1–7,400	0	0	17	50	4
18	1–6,600	0	0	18	10	2
19	1–3,330	0	0	19	5	7
20	1–2,220	0	0	20	5	2
21	1–1,665	0	0	21	5	2
22	1–1,332	0	0	22	10	3
23	1–1,110	0	0	23	100	5
24	1–951	0	0	24	50	3
25	1–830	0	0	25	50	10
26	1–740	0	0	26	5	5
27	1–660	0	0	27	50	7
28	1–333	0	0	28	100	10
29	1–222	0	0	29	20	4
30	1–166	0	0	30	25	5
31	1–133	0	0	31	27	1

No visible reduction in bacterial content of tubes containing drug as compared with controls.

TABLE 4

THE EFFECTS OF YATREN PURISSIUM ON E. DYSENTERIAE IN VITRO

No. of culture tube	Dilution of drug in medium	Number of amoebas present in cover-slip preparation		No. of control tube	Number of amoebas present in cover-slip preparation	
		After 24 hours	After 48 hours		After 24 hours	After 48 hours
1	1-10,000	110*	4*	1	30	15
2	1-5,000	40*	15*	2	17	10
3	1-3,330	10*	2*	3	25	7
4	1-2,500	16*	1*	4	45	4
5	1-2,000	0*	0*	5	50	11
6	1-1,660	1*	0*	6	27	17
7	1-1,420	1†	0†	7	31	31
8	1-1,250	1†	0†	8	39	10
9	1-1,110	0†	0†	9	100	14
10	1-1,000	0†	0†	10	15	5
11	1-500	0‡	0†	11	75	15
12	1-333	0‡	0†	12	54	12
13	1-250	0‡	0†	13	33	6
14	1-200	1 (dead)‡	0†	14	50	7
15	1-100	0§	0§	15	74	11
16	1-50	0§	0§	16	53	13

* Normal growth of bacteria.
† Bacterial growth slightly inhibited.
‡ Marked inhibition of bacteria.
§ Marked inhibition of bacteria; motility lost.

TABLE 5

THE EFFECTS OF YATREN CASEIN ON E. DYSENTERIAE IN VITRO

No. of culture tube	Dilution of drug in medium	Number of amoebas present in cover-slip preparation		No. of control tube	Number of amoebas present in cover-slip preparation	
		After 24 hours	After 48 hours		After 24 hours	After 48 hours
1	1-10,000	100*	2*	1	10	10
2	1-5,000	10*	0*	2	15	25
3	1-3,330	100*	0*	3	50	30
4	1-2,500	8*	0*	4	100	30
5	1-2,000	19*	5*	5	121	3
6	1-1,660	3*	0*	6	50	15
7	1-1,420	2*	1*	7	140	6
8	1-1,250	0*	2*	8	160	9
9	1-1,110	0*	2*	9	90	40
10	1-1,000	1*	0*	10	122	21
11	1-500	1†	4*	11	130	12
12	1-333	2†	0*	12	80	6
13	1-250	1†	1*	13	75	7
14	1-200	0†	0*	14	40	14
15	1-100	0†	0*	15	130	10
16	1-50	0†	2 (dead)*	16	50	7

* Normal growth of bacteria.
† Bacterial growth slightly inhibited.

TABLE 6

THE EFFECTS OF NEO-ARSPHENAMINE ON E. DYSENTERIAE IN VITRO

No. of culture tube	Dilution of drug in medium	Number of amoebas present in cover-slip preparation		No. of control tube	Number of amoebas present in cover-slip preparation	
		After 24 hours	After 48 hours		After 24 hours	After 48 hours
1	1–1,000,000	15	2	1	10	15
2	1–500,000	20	1	2	15	13
3	1–333,000	40	0	3	20	4
4	1–250,000	20	0	4	10	10
5	1–166,000	1	0	5	50	12
6	1–142,000	15	0	6	5	15
7	1–125,000	0	0	7	10	8
8	1–111,000	0	0	8	11	3
9	1–100,000	0	0	9	50	2
10	1–50,000	0	0	10	13	4
11	1–33,300	0	0	11	35	5
12	1–25,000	0	0	12	42	7
13	1–16,600	0	0	13	21	8
14	1–14,200	0	0	14	37	10
15	1–12,500	0	0	15	13	3
16	1–11,100	0	0	16	22	4
17	1–10,000	0	0	17	15	30
18	1–5,000	0	0	18	8	10
19	1–3,330	0	0	19	12	11
20	1–2,500	0	0	20	7	10
21	1–1,660	0	0	21	30	5
22	1–1,420	0	0	22	120	7
23	1–1,250	0	0	23	30	10
24	1–1,110	0	0	24	15	10

No visible reduction in bacterial content of tubes containing drug as compared with controls.

TABLE 7

THE EFFECTS OF ARSPHENAMINE ON E. DYSENTARIAE IN VITRO

No. of culture tube	Dilution of drug in medium	Number of amoebas present in cover-slip preparation		No. of control tube	Number of amoebas present in cover-slip preparation	
		After 24 hours	After 48 hours		After 24 hours	After 48 hours
1	1–1,000,000	50	25	1	40	12
2	1–500,000	220	20	2	125	10
3	1–333,000	150	15	3	54	3
4	1–250,000	53	30	4	45	3
5	1–166,000	100	0	5	30	15
6	1–142,000	110	1	6	273	7
7	1–125,000	30	1	7	32	3
8	1–111,000	12	0	8	35	5
9	1–100,000	7	3	9	24	7
10	1–50,000	5 (dead)	0	10	72	8
11	1–33,300	0	0	11	25	5
12	1–25,000	0	0	12	10	7
13	1–16,600	0	0	13	5	8
14	1–14,200	0	0	14	7	5
15	1–12,500	1 (dead)	0	15	10	4
16	1–11,100	0	0	16	4	11
17	1–10,000	0	0	17	7	3
18	1–5,000	0	0	18	5	3
19	1–3,330	0	0	19	10	7
20	1–2,500	0	0	20	12	10
21	1–1,600	0	0	21	30	13
22	1–1,420	0	0	22	20	7
23	1–1,250	0	0	23	15	5
24	1–1,110	0	0	24	10	4
25	1–1,000	0	0	25	10	8
26	1–500	0	0	26	30	7

No visible reduction in bacterial content of tubes containing drug as compared with controls.

TABLE 8

THE EFFECTS OF SULPHARSPHENAMINE ON E. DYSENTERIAE IN VITRO

No of culture tube	Dilution of drug in medium	Number of amoebas present in cover-slip preparation		No of control tube	Number of amoebas present in cover-slip preparation	
		After 24 hours	After 48 hours		After 24 hours	After 48 hours
1	1-1,000,000	50	50	1	25	5
2	1-500,000	2	0	2	5	10
3	1-333,000	5	50	3	2	5
4	1-250,000	0	15	4	11	10
5	1-200,000	0	17	5	4	10
6	1-166,000	1	5	6	20	8
7	1-142,000	0	0	7	30	10
8	1-125,000	0	3	8	5	15
9	1-111,000	9	20	9	30	5
10	1-100,000	1	10	10	35	7
11	1-50,000	0	27	11	45	18
12	1-33,300	12	25	12	70	23
13	1-25,000	0	5	13	47	10
14	1-20,000	3	3	14	17	8
15	1-16,600	0	2	15	20	11
16	1-14,200	0	0	16	13	4
17	1-12,500	2	0	17	117	5
18	1-11,100	0	0	18	87	27
19	1-10,000	2	0	19	15	3
20	1-5,000	0	0	20	17	5
21	1-3,330	4	0	21	10	4
22	1-2,500	0	0	22	21	4
23	1-2,000	0	0	23	13	11
24	1-1,660	0	0	24	15	7

No visible reduction in bacterial content of tubes containing drug as compared with controls.

TABLE 9

THE EFFECTS OF MERCUROCHROME ON E. DYSENTERIAE IN VITRO

No. of culture tube	Dilution of drug in medium	Number of amoebas present in cover-slip preparation		No. of control tube	Number of amoebas present in cover-slip preparation	
		After 24 hours	After 48 hours		After 24 hours	After 48 hours
1	1–100,000	25	3	1	10	2
2	1–50,000	10	0	2	25	5
3	1–33,300	25	1	3	2	10
4	1–25,000	7	0	4	20	3
5	1–16,000	0	1	5	50	5
6	1–14,200	1	1	6	10	4
7	1–12,500	1	5	7	3	5
8	1–11,100	3	0	8	3	5
9	1–10,000	0	1	9	10	3
10	1–5,000	0	1	10	3	2
11	1–3,330	0	0	11	3	3
12	1–2,500	0	0	12	10	3
13	1–1,600	0	0	13	50	10
14	1–1,400	0	0	14	2	12
15	1–1,250	0	0	15	3	10
16	1–1,000	0	0	16	25	20
17	1–500	0	0	17	10	25
18	1–333	0	0	18	35	3

No visible reduction in bacterial content of tubes containing drug above a 1–1,000 dilution as compared to the controls.

The results obtained in the preceding experiments would indicate that the amoebicidal effect of the drug used depended greatly upon the number of amoebas present in the culture. Without doubt there must be great variation in the actual number of amoebas inoculated into the different tubes in an experiment. Unfortunately, there is no way to estimate the number of amoebas inoculated into fresh media by direct count due to their irregular distribution on the surface of the egg slant. Even in controlling this error, as we have done by inoculating the tube of medium containing the dilution of the drug and a control tube at the same time with the same amount of sediment from a positive 48-hour culture, it is possible for more amoebas to be added to one tube than to the other. These factors would cause many errors in the results obtained. A second source of deviation would be in the increase of toxicity of certain of the arsphenamine compounds because of their rapid oxidation during the experiment.

With due regard for these variables in our results, it will be noted that the five arsenical compounds are highly amoebicidal in their action, sulph-arsphenamine being the least active. Emetin hydrochloride was found to be effective only up to a 1-16,000 dilution. The yatren compounds, although highly recommended in the treatment of amoebiasis, had very slight effect on *E. dysenteriae in vitro.* Hexylresorcinal was also used, but as the drug could be obtained only in 25 per cent olive oil, it floated to the surface of the culture medium and was ineffective.

It has been found that in the cultures containing the lowest dilution of the drug in which amoebas persisted (less than ten amoebas present in a cover-slip preparation), the amoebas present were highly vacuolated and were two to three times their normal size. The average size of *E. dysenteriae* in 24-hour cultures has been found to be about 11 microns in diameter (Kofoid and Wagener, 1925). This increase in size of the few surviving amoebas found in cultures at the point of toxicity of the drug would seem to indicate that they had lost their power to divide.

SUMMARY

1. The effects of ten drugs have been tested on *Endamoeba dysenteriae* by adding the drug in graded dilutions to cultures of this species of this amoeba in egg-Locke-blood medium.

2. The lethal dilution of these drugs in cultures has been found at the end of 24 hours to be as follows:

Emetin hydrochloride	1–10,000 dilution
Acetylarsan	1–10,000 dilution
Stovarsol	1–95,142 dilution
Yatren purissium	1–1,250 dilution
Yatren casein	1–250 dilution
Neo-arsphenamine	1–142,000 dilution
Arsphenamine	1–100,000 dilution
Sulph-arsphenamine	1–3,330 dilution
Mercurochrome	1–11,100 dilution.

3. In cultures containing the lowest dilution of a drug in which *E. dysenteriae* persisted, the amoebas are highly vacuolated and have failed to divide. Such amoebas are two or three times their normal size and are moribund.

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SOME NEW AND SOME PREVIOUSLY UNREPORTED HYDROIDS, MAINLY ·FROM THE CALIFORNIAN COAST

BY

C. McLEAN FRASER

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SOME NEW AND SOME PREVIOUSLY UNREPORTED HYDROIDS, MAINLY FROM THE CALIFORNIAN COAST

BY

C. McLEAN FRASER

In material collected by the United States Bureau of Fisheries Steamer "Albatross," and by the University of California, mainly from the Californian coast, sent to me for examination by Dr. C. A. Kofoid, there are seven species that appear to be new. Unfortunately, in no case was the gonosome present. Of necessity, then, some of the species can be only provisionally placed in the genus assigned. Five of the species were found in material from the Californian coast, one from off the Oregon coast, and one, rather out of place in such a collection, was taken at a "Fishhawk" station, off the Florida coast.

Besides these new species, four species, not previously reported from the Pacific coast of North America, were obtained, all from the San Francisco Bay region. As these will be described later, in a key to the hydroids of the Pacific coast of the United States, and as they have already been well described in one or more papers, only the distribution of the specimens in the collection is given here.

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? **Coryne corrugata** sp. nov.

Figure 1

Trophosome.—Colony reaching a height of 3.5 cm., much branched, *the branches coming off with a definite knee joint at a very acute angle with the stem; the secondary branches arise from the primary* branches in the same manner; many of these secondary branches, while terminating in a hydranth, give off numerous branchlets that have no hydranths; perisarc thick, with deep annulations, that may be considered as corrugations, throughout the whole length of the stem and branches; hydranths capable of great extension and usually appearing rather long and slender; tentacles 20–30, not arranged in very definite verticils.

Gonosome.—Unknown.

Distribution.—San Diego, near jetty.

Since no gonosome was present in the material examined it is not possible to say definitely whether the species is a *Coryne* or a *Syncoryne*. It bears considerable resemblance to *Coryne brachiata* Nutting, but differs from it materially in the mode of branching, and it lacks the special processes below the hydranth to which *C. brachiata* owes its name.

? **Bimeria pusilla** sp. nov.

Figure 2

Trophosome.—Colony small, straggling, less than 5 mm. high; from one to three main branches that are similar in size and appearance to the main stem; secondary branches vary in length and are irregularly placed; these may bear hydranths or may divide again to form pedicels for hydranths; the angle that the small branches make with the larger, and that the larger make with the stem, is very variable; the perisarc is thin, nowhere annulated or wrinkled, but there is a slight tendency to waviness that prevents complete smoothness; this waviness appears in the small branches as well as in the larger and the stem; hydranths with 12–14 tentacles.

Gonosome.—Unknown.

Distribution.—Lime Point, San Francisco Bay.

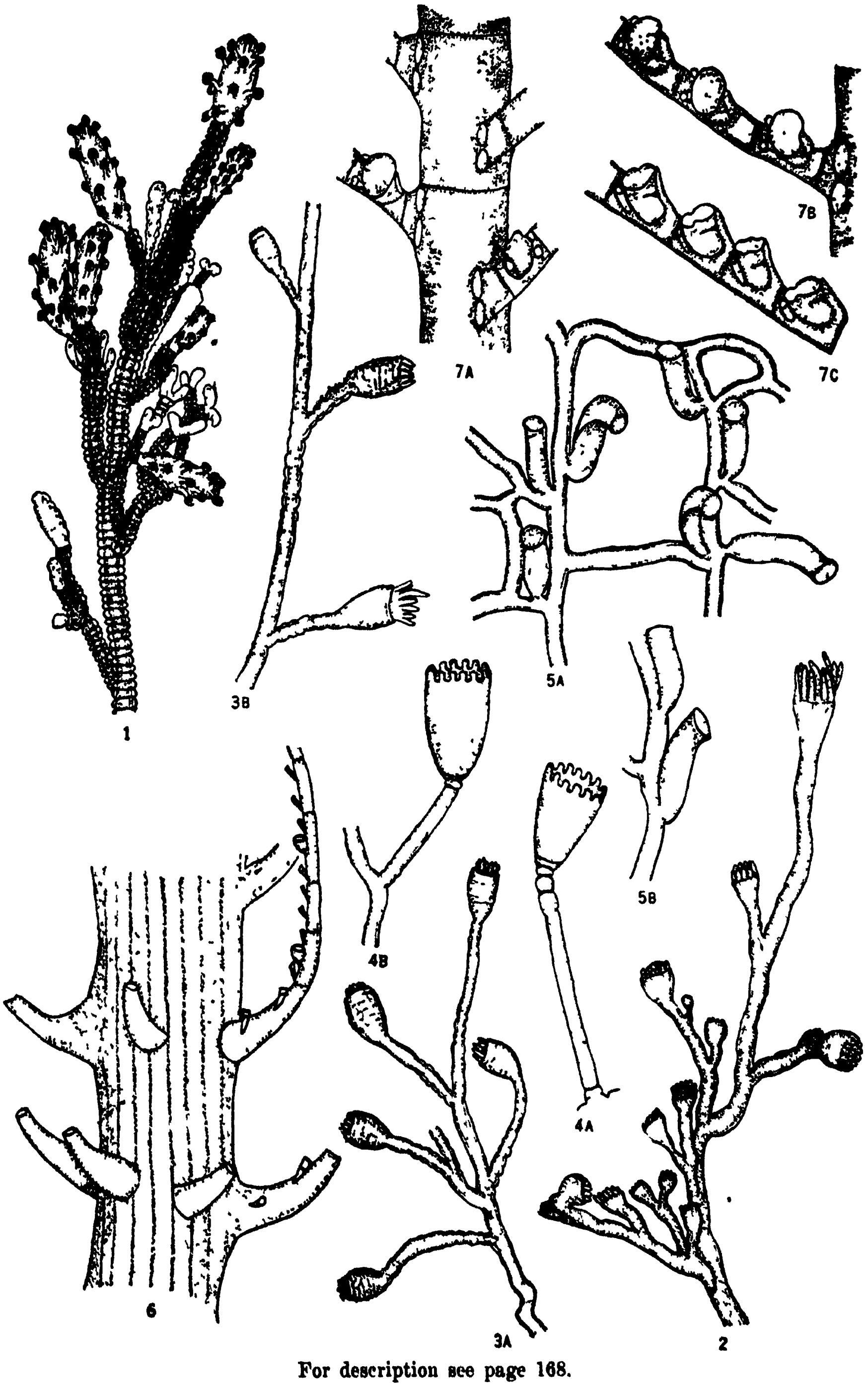
? **Bimeria tenella** sp. nov.

Figure 3

Trophosome.—Stem simple or with a slight tendency to fasciculation, reaching a height of 15 mm.; stem and branches slender, the branches making a wide angle with the stem, and the pedicels of the hydranths making a wide angle with the stem or branches; the pedicels attached to the stem in general much longer than those

Figures 1–7

1. ***Coryne corrugata* sp. nov. Hydranths and mode of branching. × 5.**
2. ***Bimeria pusilla* sp. nov. Main portion of colony. × 10.**
3. ***Bimeria tenella* sp. nov. × 10. *a* and *b*, two portions of colony.**
4. ***Campanularia castellata* sp. nov. × 10. *a* and *b*, two hydrothecae.**
5. ***Lafœa adnata* sp. nov. × 10. *a*, portion of colony showing network; *b*, separate portion of network showing two hydrothecae.**
6. ***Antennularia verticillata* sp. nov. Main stem showing arrangement of hydrocladia. × 10.**
7. ***Halicornaria sinuosa* sp. nov. × 10. *a*, portion of main stem; *b*, side view of hydrocladium; *c*, face view of hydrocladium.**



For description see page 168.

attached to the branches; the main stem and branches mainly smooth, but there may be a wrinkling or even an annulation for short distances; pedicels annulated extensively at the base or more rarely annulated or wrinkled throughout the whole length; the perisarc surrounding the base of the hydranth is heavy and somewhat rough or wrinkled; hydranths with 10 tentacles.

Gonosome.—Unknown.

Distribution.—Several points near the entrance of San Francisco Bay in the neighborhood of Southampton Light, Angel Island, and Alcatraz Island. Depth, 9–22 fathoms.

This species bears close resemblance to *Bimeria vestita* Wright, the most important difference being in the much smaller number of tentacles in the hydranth, 16 in *B. vestita.* There is some resemblance to *B. gracilis* (Clark), also, but that species is strongly fascicled and the branches and pedicels pass out at a very narrow angle so that they are almost parallel with the stem.

? **Campanularia castellata** sp. nov.

Figure 4

Trophosome.—Stem unbranched, forming the hydranth pedicel, from 0.8 mm. to 4.0 mm. in length, terminated below the hydrotheca in a ball-shaped joint, but otherwise only slightly annulated, if at all; in some cases there is a slight waviness near each extremity; stolon not annulated; hydrotheca large, 0.6 to 1.0 mm. in length and 0.4 to 0.6 mm. in greatest width, tapering but slightly from the margin to near the base, the base nearly hemispherical; the 12–14 teeth are deep, the same width throughout or slightly broader near the tip, which is just noticeably rounded; the hydrothecal wall is uniform in thickness, without lines or ridges; the space between the diaphragm and the base is shallow.

Gonosome.—Unknown.

Distribution.—Near Alcatraz Island, entrance of San Francisco Bay, 10–17 fathoms, growing on *Sertularia desmoides.*

This species bears close resemblance to *Campanularia grœnlandica* Levinsen, but as *C. grœnlandica* has such very distinct lines running vertically along the hydrotheca from the spaces between the teeth and these specimens have no indication of anything of the kind, they cannot well be included with that species.

Lafœa adnata sp. nov.

Figure 5

Trophosome.—Hydrothecae attached to a reticulate stolon, with no definite pedicels, although there is a definite constriction at the base of each; in nearly all cases the wall of the hydrotheca is attached to the stolon for a part of its length but the proportion varies very materially; except for the tapering to the constriction the hydrotheca is of uniform diameter; there may or may not be a slight flare at the margin; the hydrotheca is always curved but the amount of curvature varies; in extreme cases the distal extremity is at right angles to the proximal; there is no diaphragm present; in the specimens examined the hydranth has 8 tentacles.

Gonosome.—Unknown.

Distribution.—Near Farallon Islands in 33–35 fathoms, on algae, bryozoa, and other hydroids.

Antennularia verticillata sp. nov.

Figure 6

Trophosome.—A fragment of a stem 2.5 cm. long is stout and uniform, diameter 1.0 mm. The canaliculated appearance of the coenosarc is very regular, there being 24 vertical, parallel grooves showing at the surface; hydrocladia arranged in very regular whorls of six, the individual hydrocladia in one whorl opposite the spaces between the hydrocladia in the whorl above and the whorl below, so that there are really 12 vertical series, and thus two coenosarcal canals for each series; the basal hydrocladial internode is long, curved, and so much stouter than the remainder of the hydrocladium that it might be considered a process of the stem, bearing the hydrocladium, particularly, since it is at the distal extremity of the internode that the hydrocladium breaks off the most readily; this basal internode bears two nematophores; and next to it there is a hydrothecate internode, with one nematophore below the hydrotheca and two above it; a non-hydrothecate internode with two nematophores follows; the hydrothecate and non-hydrothecate internodes then alternate throughout the hydrocladium.

Gonosome.—Unknown.

Distribution.—31.7 miles N 75° E of Heceta Head Light, Oregon coast, 84 fathoms.

In the regular and alternating whorls of hydrocladia and in the nature of the proximal internodes of the hydrocladia, this species is distinctly marked off from other species that have been described.

No species of the genus *Antennularia* has previously been reported from the Pacific coast of North America.

Halicornaria sinuosa sp. nov.

Figure 7

Trophosome.—Colony unbranched, largest obtained 12 cm. in length; stem simple, divided into regular internodes, each of which bears two hydrocladia, placed on the one side of the stem at an angle of 135° or less, the one higher than the other on the internode, but the two on the one internode nearer together, in a vertical direction, than the nearest two in succeeding internodes; hydrocladia divided into short internodes, all hydrothecate; hydrothecae with length and breadth nearly equal or slightly deeper than broad, but slightly curved, hence running almost parallel to the axis of the hydrocladium; the margin, which is sinuous, not toothed, making an angle of about 45° with the axis; the intrathecal ridge is strongly marked; mesial nematophore straight, just reaching the margin of the hydrotheca; the two supracalycine nematophores reaching the margin of the hydrotheca; two large cauline nematophores on the face of the stem at the base of each hydrocladium, and one, also large, at the back of the same process.

Gonosome.—Unknown.

Distribution.—Fishhawk Station 7511, 2⅛ miles SSE of Fowey Rock Light, Gulf Stream off Cape Florida, 45 fathoms. With the hydroids *Aglaophenia rigida* and *Schizotricha tenella.*

Turritopsis nutricula McCrady

Oceania nutricula McCrady, Proceedings Elliott Soc., **1856**, 1–56.

Turritopsis nutricula McCrady, Gymnoph. Charleston Har., **1857**, 25.

Turritopsis nutricula Fraser, Hydroids of Beaufort, **1912**, 345.

Distribution.—Oakland, California.

Pennaria tiarella McCrady

Pennaria tiarella McCrady, Gymnoph. Charleston Har., **1857**, 51.

Pennaria tiarella Fraser, Hydroids of Beaufort, **1912**, 355.

Distribution.—San Francisco Bay at entrance of San Pablo Bay, 4 fathoms.

Obelia bicuspidata Clarke

Obelia bicuspidata Clarke, Trans. Conn. Acad. Sci., **3**, 1876, 58.

Obelia bicuspidata Fraser, Hydroids of Beaufort, **1912**, 361.

Distribution.—Generally distributed throughout San Francisco Bay, from 7 to 12 fathoms; at the entrance to Carquinez Strait, at the entrance to San Pablo Bay, near Southampton Light and Alcatraz Island, and near Shag Rock in the lower bay.

Cryptolaria pulchella Allman

Cryptolaria pulchella Allman, Challenger Hyd., **23**, 1888, 40.

Distribution.—Off Goat Island, San Francisco Bay, 10 fathoms.

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THE EFFECT OF CERTAIN DRUGS AND DYES UPON THE GROWTH OF ENDAMOEBA GINGIVALIS (GROS) IN VITRO

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CONTENTS

PAGE
Introduction and acknowledgments 173
Methods 174
Experiments:
  Arsenical compounds 176
  Non-arsenical compounds 177
  Dyes 178
Summary 179
Literature cited 182

INTRODUCTION AND ACKNOWLEDGMENTS

It has been shown by Drbohlav (1925*b*) and Howitt (1925) that *Endamoeba gingivalis* (Gros), the amoeba of the mouth, can be readily grown under artificial conditions. The following experiments were undertaken to determine the effect of certain drugs and dyes upon the growth of the organism *in vitro*.

The work was carried on under the direction of Professor Charles A. Kofoid at the University of California and was made possible by grants to the California Stomatological Research Group from the Carnegie Corporation, from the American Dental Association, from the Associated Radiograph Laboratories of San Francisco, and from the California State Dental Association.

METHODS

The medium used for the cultivation of *E. gingivalis,* together with its method of preparation, has already been described elsewhere (Howitt, 1925), being a slight modification of the Locke-albumen egg slant medium used by Boeck and Drbohlav (1925) and Drbohlav (1925*a*) for the cultivation of *E. dysenteriae* (*histolytica*). Throughout the present experiments, however, the Locke-albumen mixture was passed through a Berkefeld filter and contained the white of only one egg to a liter of Locke's, instead of the whites of two eggs as previously used. The reaction was adjusted to between pH 7.4 and pH 7.6.

In preparing the different series of substances used in the experiments, the following technique was employed as a general routine. Ten c.c. amounts of the Locke-albumen mixture (L. A. medium) were added aseptically with a sterile pipette to a series of coagulated egg slant tubes, the number of tubes corresponding to the number of drug dilutions to be made. When the solubility of the substance permitted, the drug or dye to be used was prepared as a 10 per cent solution, from which dilutions of 1:100 and 1:1000 might be made if necessary.

These solutions were added with a graduated 0.1 c.c. pipette to the tubes containing the 10 c.c. of Locke-albumen medium, in such amounts as to make the graded series of dilutions shown in table 1. Both the dilutions of the material used and its actual percentage in the tubes are given in the table. A number of control Locke-albumen egg slant (L. E. A.) tubes without any drug were prepared at the same time.

Approximately equal numbers of drops from six or seven active, 24-hour L. E. A. cultures of *E. gingivalis* were then added with a capillary pipette to each of the experimental and the control tubes. As a rule, there was one L. E. A. control for every two experimental tubes, so that one could easily judge of the activity of the stock culture. While this method of inoculation was not strictly quantitative, since it was not certain how many amoebae went into each culture, the results were fairly comparable, especially when checked against the controls for each particular tube.

The inoculated cultures were placed in the incubator at 37° C and observed every 24 hours for about three days. The number of amoebae in each tube was counted according to the method previously described (Howitt, 1925) and a comparison of the results for all the substances used is shown in table 1. The small italicized letters placed after the numbers in the first section refer to the control L. E. A. tube of the same letter for each particular substance. Letter "*a*" for example, in the first dilution column for stovarsol refers to the L. E. A. control "*a*" of the same drug found at the extreme right of the table on the stovarsol line. It is thus possible to find all the control tubes for the dilutions of each drug.

The drugs used in this work, emetin hydrochloride, stovarsol, arsphenamine, neo-arsphenamine, sulfarsphenamine, acetylarsan, and yatren, are some of those that have recently been employed as therapeutic agents against *Endamoeba dysenteriae*, the pathogenic amoeba of the bowel. Emetin has been especially successful against *E. dysenteriae* and was also considered effective against *E. gingivalis* during that period when Bass and Johns (1915) and Smith and Barrett (1915) endeavored to show that this organism was the causative agent of pyorrhoea alveolaris. There was also a belief among certain workers that the mouth amoeba might be the same as that of the bowel. It was found later, however, that emetin did not affect a permanent cure for pyorrhoea, so that the relationship of *E. gingivalis* to this disease was discredited. Recently Kofoid and Swezy (1924) have shown that the two amoebae are separate species, and it need not be expected, therefore, that they would be similarly affected by the same drugs. Neither is it to be expected that the treatment of *E. gingivalis* with the different drugs *in vitro* is exactly comparable to the treatment of the organism with the same drugs *in vivo*. There are too many unknown factors to be considered before attempting any arbitrary statements, and one can gain only a very general, although somewhat comparative, idea of the behavior of the organism toward each substance.

All of the experiments, except one of the emetin series, were performed with cultures of *E. gingivalis* from strain No. 38, which was obtained from a deep gingival pocket on February 26, 1925, and has been carried through eighty-six transplantations to the date of writing (August, 1925). The second emetin series was performed with strain No. 44, obtained from the mouth of a person having only a slight case of gingivitis. The culture was in its thirteenth generation at the time the experiment was carried out.

EXPERIMENTS

I. Arsenical Compounds

(*a*) *Stovarsol.*—Stovarsol (acetyl-oxy-amino-phenyl-arsenic acid) has been used recently by the French for the treatment of both syphilis and amoebic dysentery. Marchoux (1922) reported it successful in several cases of dysentery which had proved refractory to emetin and several other workers have reported it equally satisfactory (Manson-Bahr, 1925). The drug is prepared in 0.25 gm. tablets composed of some powdery substance which forms an insoluble suspension when diluted with Locke's solution. The drug itself is soluble in water, so that, if the suspension is well shaken and rapidly added to the tubes of L. E. A. medium, fairly accurate dilutions may be obtained.

The effect of the different dilutions of stovarsol upon *E. gingivalis in vitro* may be seen in table 1. The lethal dose was 1–33,300 (0.003 per cent stovarsol), although the organisms were undoubtedly somewhat reduced in numbers in the several preceding dilutions, especially in 48 hours. This reduction in amoebae is apparent from a comparison with the number of active organisms found in the control L. E. A. tubes.

(*b*) *Arsphenamine and its derivatives neo-arsphenamine and sulfarsphenamine.*—While arsphenamine and its derivatives, neo-arsphenamine and sulfarsphenamine, are readily soluble in water, they are also easily oxidized, especially in the presence of heat, so that the dilutions must be used immediately. In spite of precautions, the toxicity of these compounds for *E. gingivalis in vitro* was undoubtedly greater than would be expected *in vivo.*

Both acid and neutralized arsphenamine were used, the former being more toxic than the latter and never used therapeutically. The experimental lethal dose for the acid form was 1:25,000 (0.004 per cent arsphenamine) while that for the other lay between 1:16,600 (0.006 per cent) and 1:12,500 (0.008 per cent). The neutralized drug, however, was undoubtedly quite toxic since, compared to the numbers in the control tubes, very few organisms were found in dilutions greater than 1:100,000. Amoebae were present in a 1:100,000 dilution in the acid series but rapidly died off in the succeeding dilutions. They had also disappeared from all the tubes in 48 hours, showing that the toxicity increased with the time factor.

Neo-arsphenamine and sulfarsphenamine can be used without further neutralization. In each case the lethal dose was 1:25,000 (0.004 per cent), the same as with acid arsphenamine. Neo-arsphenamine, however, was slightly more toxic than the others, since there were fewer organisms present in the tubes with similar dilutions of drug.

(*c*) *Acetylarsan.*—Acetylarsan (oxy-acetyl-amino-phenyl arsinate) is another arsenical compound used recently by certain French workers (Mahoux, 1923) for the treatment of syphilis. It is put up in 3 c.c. ampoules containing 0.23 per cent of the active drug, so that it is easily diluted to the required strength. This drug was slightly less effective than the other arsenicals used, the lethal dose being a 1–14,200 dilution (0.007 per cent acetylarsan). Very few organisms were found, however, in the three or four preceding dilutions in 24 hours, and in 48 hours they had disappeared.

II. NON-ARSENICAL COMPOUNDS

(*a*) *Emetin hydrochloride.*—Emetin hydrochloride is a soluble salt so that it is easily prepared in a 10 per cent solution. Two series of experiments were carried out with this drug, one against culture No. 38 of *E. gingivalis* and the other against culture No. 44. There was no very striking difference in the results from either culture, although the lethal dose for No. 38 (1:2500 or 0.03 per cent emetin) was in a slightly higher dilution than that for No. 44 (1:1420 or 0.07 per cent emetin). Although the organisms began to appreciably diminish in concentrations greater than 1:16,600, especially in 48 and 72 hours, yet this drug was not nearly so effective toward *E. gingivalis* as the arsenic compounds first described.

(*b*) *Yatren purissimum.*—Yatren purissimum ($C_9H_6O_4SNI$) is a German product composed of 28 per cent iodine with oxy-quinoline-sulphonic acid and about 20 per cent $NaHCO_3$ (Manson-Bahr, 1925). It is soluble in warm water at 80° C with the liberation of CO_2, so that care must be taken to avoid losing some of the substance through evanescence.

The drug has been used as an antiseptic and as an intestinal lavage for amoebic dysentery, being administered in the latter case in 0.25 gm. (4 gr.) pills by mouth or as an enema, 3–5 gm. in 200 c.c. water. When used experimentally against cultures of *E. gingivalis*, yatren was undoubtedly the least effective of any of the substances tried. Excellent growth and multiplication of amoebae occurred in all dilutions as low as 1:1420 (0.07 per cent yatren), when the organisms first began to appreciably disappear. The lethal dose was about 1:1000 (0.1 per cent yatren), although one amoeba was noticed in a dilution of 1:500.

III. DYES

Since these experiments were not performed on bacteria-free cultures, it was somewhat difficult to estimate the exact effect upon the amoebae alone. It was thought of interest, therefore, to try the effect of the two dyes, acriflavine and gentian violet, on the cultures of *E. gingivalis,* in order to find the strongest dilution at which the amoebae would live and multiply, with the hope that repeated cultivation in that concentration of dye might finally reduce the bacteria and permit the amoebae to thrive alone. These two dyes were chosen because acriflavine is known to possess an increased activity in the presence of albuminous material and because gentian violet is considered to have a bactericidal action against gram positive organisms, such as predominate in these cultures from the buccal cavity.

(*a*) *Acriflavine.*—Acriflavine (diamino-methyl-acridinium) is an acridine compound whose bactericidal action is greatly increased both by the presence of serum and by an increase in the pH of the medium from 4.0 to 11.0 (Browning, Gulbransen, and Kennaway, 1919). The presence of the egg white in the medium would not therefore inhibit the activity of the dye.

Acriflavine proved to be quite toxic for the cultures of *E. gingivalis* as well as for the bacteria, since the amoebae first began to appreciably diminish at a dilution of 1:111,000 (0.009 per cent dye). Only a few organisms were found in the succeeding dilutions until the lethal dose of 1:33,300 was reached. The dye would have to be used, therefore, in a very high dilution, either 1:142,000, or 1:125,000 in order not to inhibit the growth of the amoebae.

(*b*) *Gentian violet.*—Gentian violet was found by Churchman (1912) to be a bactericidal agent against the gram positive bacteria but not against the gram negative. It might therefore be of some use in an attempt to eliminate the dominant gram positive flora from these cultures of *E. gingivalis.*

The amoebae could withstand very much stronger concentrations of this dye than they could of acriflavine, since a few organisms were present even in a dilution of 1:10,000, beyond which concentration they disappeared from the cultures. They began to diminish in the successive dilutions following 1:50,000, but the latter concentration is probably the most favorable one to use, since the bacteria are reduced while the amoebae continue to flourish.

SUMMARY

In table 2 may be found a comparative summary of the end results of these different experiments, giving (1) the dilution at which the amoebae first began to be appreciably diminished; (2) the strongest concentration showing amoebae to be present; and (3) the lethal dose. It is evident that the arsenical compounds, with the exception of acetylarsan, are the most toxic of the drugs, although it must be taken into account, that their readiness to undergo oxidation may have somewhat increased the degree of toxicity in each case. Of the arsenicals, stovarsol is apparently the most effective and acetylarsan the least, although there is no very marked difference in their toxicity.

Emetin hydrochloride has apparently no very marked amoebicidal action *in vitro* against either of the strains of *E. gingivalis* used, since a few amoebae were able to survive as strong a concentration as 1:1660 (0.06 per cent). The organisms, however, were appreciably diminished after a dilution of 1:25,000, which was usually the lethal dose for the arsenic drugs. While amoebae were present in these stronger concentrations, yet their numbers were greatly reduced as compared to those present in the control tubes. No multiplication took place and the cultures were usually negative in 48 or 72 hours. Emetin, therefore, must have had a certain amount of effect, killing off the weaker forms and allowing the hardier ones to survive for at least 24 hours. Kofoid and Wagener (1925) have found somewhat similar results when using both emetin and the aforementioned arsenicals against *E. dysenteriae in vitro.*

Yatren is undoubtedly the least effective of any of the drugs tried, abundant growth being present in dilutions to 1:2000, after which they gradually diminished. The dye, acriflavine, seems fully as toxic for *E. gingivalis in vitro* as the arsenic compounds so that it

TABLE 2

TABLE COMPARING THE END RESULTS OF THE DRUG DILUTION EXPERIMENTS

Drug or dye	Highest dilution showing a marked reduction in amoebae			Lowest dilution showing some amoebae			Lethal dilution		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Stovarsol (June 22)	1-125,000	1-142,000	....	1-50,000	1-50,000	....	1-33,300	1-33,300	....
Sulfarsphenamine	1-33,300	1-142,000	1-166,000	1-33,300	1-100,000	1-166,000	1-125,000	1-50,000	1-142,000
Neo-arsphenamine	1-100,000	1-125,000	....	1-33,300	1-100,000	1-125,000	1-25,000	1-50,000	1-111,000
Acid arsphenamine	1-50,000	Negative	....	1-33,300	Negative	....	1-25,000	Negative	....
Neutralized arsphenamine	1-100,000	1-50,000	1-100,000	1-14,200	1-33,300	1-50,000	1-12,500	1-33,300	1-50,000
Acetylarsan	1-33,300	1-50,000	....	1-16,600	1-50,000	....	1-14,200	1-33,300	....
Emetin hydrochloride (culture No. 38)	1-20,000	1-50,000	1-33,300	1-3330	1-20,000	1-20,000	1-2500	1-5000 1-11,100	1-16,600
Emetin hydrochloride (culture No. 44)	1-20,000	1-25,000	1-25,000	1-1660	1-12,500	1-14,200	1-1420	1-2000	1-12,500
Yatren	1-1420	1-1660	1-1660	1-500	1-1110	1-1420	1-1110 1-330	1-1000	1-1250
Acriflavine	1-111,000	1-143,000	1-142,000	1-25,000	1-50,000	1-100,000	1-20,000	1-33,300	1-100,000
Gentian violet	1-33,300	1-33,300	1-11,100	1-10,000	1-10,000	1-11,100	1-5000	1-5000	1-10,000

must be used only in very high dilutions, which are not so effective against the bacteria. Gentian violet, on the other hand, can be tolerated by the amoebae in fairly strong concentrations, so that repeated cultivation in a dilution of either 1:50,000 or 1:33,300 might eventually reduce the bacteria to a minimum.

As a general summary of this experimental work, it may be stated, with certain reservations for the inaccuracies involved, (1) that stovarsol is the most effective of the substances used *in vitro* against cultures of *E. gingivalis* and yatren the least effective; (2) that the arsenical compounds are more toxic than the non-arsenicals; (3) that emetin hydrochloride, although somewhat toxic for the amoebae, is nevertheless not specifically effective *in vitro;* and (4) that of the dyes, acriflavine and gentian violet, the former can be used only in the high dilutions because of its toxicity, while the latter may be used in very much stronger concentrations without greatly hindering the amoebic growth.

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EXPERIMENTS WITH ENDAMOEBA GINGIVALIS (GROS) IN MIXED BACTERIAL CULTURES; IN FILTERED SALIVA; ON A SOLID BASE; WITH PERITONEAL CELLS; AND WITH DIGESTIVE SECRETIONS

BY

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EXPERIMENTS WITH ENDAMOEBA GINGIVALIS (GROS) IN MIXED BACTERIAL CULTURES; IN FILTERED SALIVA; ON A SOLID BASE; WITH PERITONEAL CELLS; AND WITH DIGESTIVE SECRETIONS

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CONTENTS

PAGE

Introduction and acknowledgments 183

Methods 184

Experimental:

- (*a*) Growth of *Endamoeba gingivalis* in filtered saliva 184
- (*b*) Growth of *E. gingivalis* in media having a solid base, either of coagulated blood serum or of agar 186
- (*c*) Behavior of *E. gingivalis* toward guinea pig peritoneal cells 189
- (*d*) Effect of gastric and duodenal fluids and of bile upon *E. gingivalis* in vitro 193

Summary 199

Literature cited 199

Explanation of plates 200

INTRODUCTION AND ACKNOWLEDGMENTS

It has been shown in a previous paper (Howitt, 1925) that *Endamoeba gingivalis*, the amoeba of the mouth, can be easily cultivated under artificial conditions. Drbohlav (1925*a*, *b*) has also recently reported the growth of this organism *in vitro*. Since this earlier work (Howitt, 1925) has shown the feasibility of applying experimental methods to cultures of *E. gingivalis*, even though they are not bacteria-free, further observations were undertaken to determine certain points not previously touched upon.

The following work was carried out under the direction of Professor Charles A. Kofoid of the University of California and was made possible through grants to the California Stomatological Research Group from the Carnegie Corporation, from the American Dental Association, from the Associated Radiograph Laboratories of San Francisco, and from the California State Dental Association.

METHODS

The routine medium used in the cultivation of *E. gingivalis* was a slight modification of the Locke-albumen-egg slant medium employed by Boeck and Drbohlav (1925) in the culture of *E. dysenteriae.* The formula and details of preparation have been described elsewhere (Howitt, 1925). In the present work, however, the Locke-albumen mixture contained the white of one egg to a liter of Locke's solution, instead of the two whites formerly used. The mixture was passed through a Berkefeld filter and the reaction adjusted to between pH 7.4 and pH 7.6. Very excellent growth of amoebae was then obtained when this solution was added in 8 to 10 c.c. amounts to the coagulated egg slant medium. The previously described methods for counting the number of amoebae and for testing the pH of the medium were again employed in the present experiments, so their details need not be repeated.

Five cultures, Nos. 13, 22, 38, 42, and 44, obtained from three different people, were used in the following experiments. Nos. 13, 22, and 44 were from the mouth of a person having only a slight gingivitis; No. 38 was from material taken from a deep pus pocket, and No. 42 was from a person having a moderate case of pyorrhoea. All the cultures were active and vigorous. No. 44 had undergone about 10 transplantations, Nos. 13 and 22 between 20 and 25, and No. 38 from 40 to 50 transplantations before being used in the present experiments.

EXPERIMENTAL

(*a*) Growth of *Endamoeba gingivalis* in Filtered Saliva

Since *E. gingivalis* is found so frequently and in such large numbers in the mouths of different people, it seemed probable that human saliva must contain substances that are favorable to its development. In order to determine whether or not the amoebae would thrive in pure saliva unmixed with mucus or other foreign substances, the organisms were inoculated into a medium containing saliva which had been passed through a Berkefeld filter. The material was obtained from two sources, saliva No. 1 (table 1) being from a person whose mouth harbored *E. gingivalis,* and saliva No. 2 (table 1) being from one whose mouth was amoeba-free.

The water-clear, filtered saliva varying in reaction between pH 7.0 and pH 7.6 was added in 6 or 7 c.c. amounts both to tubes of the coagulated egg slant medium and tubes without the egg. Four separate series of saliva cultures were inoculated on different occasions with the several strains of amoebae as shown in table 1. Observations were usually made on each tube every day for about three days, both the pH of the medium being recorded and the number of amoebae counted.

TABLE 1

CULTIVATION OF ENDAMOEBA GINGIVALIS IN FILTERED HUMAN SALIVA

Series	Culture number	Medium	Initial pH	24 hours		48 hours		72 hours	
				pH	Number of amoebae	pH	Number of amoebae	pH	Number of amoebae
No 1 Planted 6-3-25	38	Saliva (1), E S *	7.5	6.2	5	6.4	129	6.8	56
		L E A † Control	7.8	5.2	110	5.8	350	5.8	215
	42	Saliva (1), E S	7.5	6.2	275	6.8	30	7.4	2
		L E A Control	7.8	6.0	100	6.4	70	7.2	9
	44	Saliva (1), E S	7.5	6.2	9	6.4	60	7.0	9
		L E A Control	7.8	5.2	30	6.0	29	6.2	8
No 2 Planted 6-15-25	38	Saliva (2), E S	7.6	6.2	300	6.6	320		
		L E A Control	7.6	5.4	50	6.2	310		
	42	Saliva (2), E S	7.6	6.4	20000–	7.0	200		
		L E A Control	7.6	6.2	1500–	7.0	900		
	44	Saliva (2), E S	7.6	6.4	400	6.8	48		
		L E A Control	7.6	5.4	200	6.2	97		
No 3 Planted 6-22-25	38	Saliva (1), E S	7.6	6.6	13	6.8	5		
		Saliva (1), no E S	7.6	7.0	0	7.2	0		
		Saliva (2), E S	7.0	6.0	400	6.8	1000		
		Saliva (2), no E S	7.0	6.6	3	6.8	0		
		L E A Control	7.6	5.2	110	5.4	520		
No 4 Planted 7-23-25	38	Saliva (2), E S	7.4	6.4	400	6.6	200	7.0	136
		Saliva (2), no E S	7.4	7.2	0	7.0	0	7.0	0
		L E A Control	7.2	5.5	160	6.0	800	7.2	176

* E S = Egg slant † L E A = Locke-egg slant-albumen

As may be seen from the table all the strains of *E. gingivalis* grew and multiplied in the filtered saliva so long as a solid base of coagulated egg was present in the tube. No growth was obtained in the cultures lacking the egg except in one instance when three amoebae survived for 24 hours. They had disappeared in 48 hours. This failure to grow in the absence of a substrate was in accordance with earlier observations (Howitt, 1925), when it was noticed that *E.*

gingivalis needed a solid substance upon which to creep, either of coagulated egg or of flakes of egg white. In all the experimental tubes containing saliva the pH of the medium was higher than in the control L.E.A. tubes, and a fine whitish precipitate was usually noticed especially along the sides of the tube. This was probably a deposit of some form of calcium. It grew heavier and darker with increasing time, and as it increased and the pH became higher, the amoebae gradually declined in numbers.

These results make it evident that different strains of *E. gingivalis* may be easily cultivated in filtered human saliva whether or not the latter is obtained from a mouth harboring this amoeba, provided a solid substrate, such as the coagulated egg medium, is present in the tube. The physiological constituents necessary for the growth of the amoeba are evidently present in the filtered saliva, so that, given the substrate present in a gingival pocket or along the margins of a tooth, it can be well understood why *E. gingivalis* flourishes so readily in the human mouth.

(*b*) Growth of *Endamoeba gingivalis* in Media Having a Solid Base, Either of Coagulated Blood Serum or of Agar

The results of using substances other than the coagulated egg for a solid substrate are given in table 2. Two series of cultures were prepared, one on May 29, 1925, and the other on June 15. In the first series the usual Locke-albumen mixture was added to tubes containing slants of both rabbit and human coagulated serum. Inoculations with amoebae were made from both strain No. 38 and strain No. 44. The same medium was repeated in the second series but, in addition, tubes were used containing slants of coagulated rat serum and of both infusion and plain agar.

All the slants of coagulated serum were made according to the formula for Loeffler's medium, except that the glucose broth of the latter was replaced by Locke's solution. Each medium was slanted and sterilized in the autoclave at 15 lbs. pressure for twenty minutes, the valves being closed to prevent the presence of air bubbles. The slants of infusion agar were prepared according to the usual method, while those of the plain agar contained 0.65 per cent NaCl and 1.5 per cent agar. The reaction in both cases was adjusted to pH 7.0. A tube containing coagulated egg and sterile distilled water, instead of the L.A. mixture, was also added to this series. Control L.E.A. cultures were always prepared for each experiment.

As shown in the table the two strains of *E. gingivalis* grew abundantly in the tubes containing serum from both the rabbit and the rat, but did not thrive so well in the tubes with human serum. This reduction of growth in the latter cultures may have been due to the fact that the human serum, although passed through a Berkefeld filter, was a pooled mixture of serums which were a few days old.

TABLE 2

CULTIVATION OF ENDAMOEBA GINGIVALIS ON COAGULATED SERUM AND ON AGAR

Series	Culture number	Medium	Initial pH	24 hours		48 hours		72 hours	
				pH	Number of amoebae	pH	Number of amoebae	pH	Number of amoebae
No. 1 Planted 5-29-25	38	L.A.,* rabbit serum slant.	8 2	6 4	555	7 0	411	7 4	164
		L.A., human serum slant.	8 2	6 4	0	7 0	0		
		L.E.A. Control	8 2	5 5	25	5 8	40	6 0	150
	44	L.A., rabbit serum slant.	8 2	6 4	430	7 8	35	7 8	10
		L.A., human serum slant.	8 2	6 4	72	7 6	4	7 6	0
		L.E.A. Control	8 2	5 4	160	5 8	15	6 0	37
No. 2 Planted 6-15-25	38	L.A., rabbit serum slant.	7 6	6 2	60	6 8	480		
		L.A., rat serum slant	7 6	6 0	275	6 2	225		
		L.A., human serum slant.	7 6	6 0	95	7 0	16		
		L.A., infusion agar slant.	7 6	6 2	230	6 8	220		
		L.A., plain agar slant (1)	7 6	5 4	0	5 4	10	5 2	1
		L.A., plain agar slant (2)..	7 6	5 4	0	5 4	2	5 2	0
		Water, egg slant	7 6	0			0		
		L.E.A. Control	7 6	5 4	50	6 2	310	6 2	140
	44	L.A., rabbit serum slant.	7 6	6 4	360	7 6	27		
		L.A., rat serum slant	7 6	6 2	600	7 0	67		
		L.A., human serum slant	7 6	6 4	44	7 6	18		
		L.A., infusion agar slant.	7 6	6 2	200	6 8	145		
		L.E.A. Control	7 6	5 4	200	6 2	97		

* L.A. = Locke-albumen.

The rat serum contained much visible fat but it is uncertain whether or not the presence of this lipoid was correlated with the excellent growth obtained, since the rabbit serum, lacking a large amount of fat, gave equally as good results. Excellent cultures were also obtained when infusion agar was used as a substrate, in which case a few small flakes of the coagulated egg may have been carried over in the inoculation; otherwise the lipoidal substances were reduced to a minimum in the fusion agar.

On the other hand, although favorable reports have been made by Drbohlav (1925) for the growth of *E. gingivalis* on a substrate containing merely agar and NaCl, yet only a scanty growth was obtained when this medium was tried with the present organisms. Cultures of this agar and L.A. medium were inoculated on three different occasions, with positive results in only the one instance. This failure of growth may have been due partly to lack of nutriment in the agar and partly to the following mechanical factor. On a previous occasion, when attempting to use slants of infusion agar, it had been noticed that the slants were not adhering to the sides of the test tubes, but could be easily moved about by agitating the fluid medium above. A very slippery surface was thus offered so that the amoebae probably slid off the agar to the bottom of the tubes where they soon died through lack of a suitable substrate. When the infusion agar was again used and slanted in such a manner that the edges were firmly adherent to the sides of the test tube forming a fairly rough surface, excellent cultures were obtained as shown in table 2. The same type of slippery surface was probably present in the earlier cultures with the plain agar when no organisms were found. In the third attempt, when more care was taken to insure a less slippery surface, a few amoebae were present.

The favorable results from the use of the coagulated egg slants may therefore be largely due to the fairly rough surface and general adherence of the medium to the test tube, since some of the egg often coagulates around the edges, forming a slight cup-like depression at the butt of the slant. It is also true that the amoebae show a decided preference for the lipoidal yolk bodies found in the egg slants, since these bodies are often ingested in large numbers.

No amoebae were noticed in the tube containing coagulated egg with sterile distilled water, showing that water alone is not an isotonic solution for the growth of *E. gingivalis,* even though the requisite substrate be supplied.

In summarizing these results it seems evident that, given a sufficiently roughened and not too slippery surface, *E. gingivalis* will grow on other substances than the coagulated egg medium for a solid base. The amoebae grew well in the presence of coagulated rabbit and rat serum, less luxuriantly with coagulated human serum, while growth was obtained with slants of infusion agar provided the surface was adherent to the sides of the test tube and not too slippery. Plain agar slants were not so successful, nor was growth obtained when sterile distilled water was added to the coagulated egg medium.

(*c*) Behavior of *Endamoeba gingivalis* Toward Peritoneal Cells of the Guinea Pig

It has been previously shown (Howitt, 1925) that *E. gingivalis* is capable of readily ingesting both human and animal red blood corpuscles. Since white blood cells are often plentiful in the human mouth, especially in cases of pyorrhoea, and since *E. gingivalis* when taken directly from the mouth usually contains ingested bodies greatly resembling leucocytes (Kofoid and Swezy, 1924), it was thought of interest to watch also the behavior of these amoebae toward a concentrated suspension of white blood cells.

A sterile leucocytic exudate was artificially induced by injecting 10 c.c. of sterile broth intraperitoneally into a guinea pig. About sixteen hours later another 10 c.c. of broth were injected. After waiting an hour the animal was killed and the profuse peritoneal exudate was removed aseptically into two centrifuge tubes with a sterile pipette. Both tubes were immediately centrifugalized and the supernatant fluid removed. To tube No. 1 was added sterile water in order to hemolize any erythrocytes, while to tube No. 2 was added sterile physiological NaCl solution to prevent hemolysis. Both tubes were centrifugalized and washed three times, one with water and the other with NaCl solution. The supernatant fluid was removed from each tube, and found to have a concentrated precipitate of white cells, chiefly polymorphonuclear leucocytes intermixed with a few lymphocytes, macrophages, and tissue cells. The following observations were then made (see tables 3 and 4).

Table 3 gives the results of direct microscopic observations upon the behavior of strains No. 38 and No. 44 when placed in contact with a drop of material from each of the tubes of peritoneal exudate. In each case a drop of the amoebic culture was mixed on a slide with a drop of exudate, taken either from the tube containing hemolyzed erythrocytes or from the one in which they were unhemolyzed. A cover-glass was applied, the edges sealed with paraffin, and the whole slide was kept at 37° C., either on the warm stage or in the incubator, observations being made at different intervals for several hours. After removing the cover glass each slide was then fixed and stained either by the iron-haematoxylin method or by Wright's stain. The latter was used in order to bring out the granulations of the leucocytes should the latter be found ingested by the amoebae.

Although red blood corpuscles were found adhering to the posterior end of several amoebae at the period of earliest observation

(10 minutes) in the preparation from the non-hemolyzed exudate, yet no red cells seemed to be actually engulfed. Clusters of seven or eight erythrocytes would often follow after an amoeba as if glued to it by some adhesive substance. Occasionally a leucocyte would be added to this trailing cellular mass. After about fifteen minutes, however, inclusions resembling leucocytic nuclei were noticed in the preparations from both strains with the non-hemolyzed exudate. A few red blood cell remains were also seen within the amoebae. Within an hour and a half all the slides showed amoebae with ingested cells, either red or white blood corpuscles, and occasionally both. As a rule the preference seemed to be given to the erythrocytes, especially in the preparations where they were unhemolyzed, although even on the slides where they were supposedly hemolyzed, shadowy erythrocytic

TABLE 3

FRESH SLIDE PREPARATIONS OF E. GINGIVALIS TREATED WITH WASHED, GUINEA PIG PERITONEAL CELLS

	Culture No. 38		Culture No. 44	
	Time	Observations	Time	Observations
RBC not hemolyzed	1:58 P.M.	Slide prepared	4:10 P.M.	Slide prepared.
	2:10	Many amoebae with adhering RBC[*], not ingested.	4:20	Many RBC adhering to amoebae.
	2:15	One amoeba with 2 included WBC[†]	4:25	One amoeba with 2 inclusions, hard to say RBC or WBC.
	2:25	WBC becoming more granular in the above amoeba.	4:27	Another amoeba found with 2 inclusions, probably leucocytes.
	3:30	Several amoebae with included WBC. Some with ingested crenated RBC.	4:45	30 amoebae present and 13 had inclusions, mainly RBCs. One organism with 9 ingested cells.
		Slide stained with iron haematoxylin showed 2 amoebae with included leucocytes.	5:30	Still 13 amoebae with inclusions. One with 14 ingested cells, hard to identify.
		Another slide treated with Wright's stain showed a few amoebae with ingested WBCs.		Slide treated with Wright's stain showed 2 amoebae with ingested WBCs.
RBC hemolyzed	1:50 P.M.	Slide prepared	4:05 P.M.	Slide prepared.
	2:00	No amoebae with ingested cells....	5:05	3 amoebae with inclusions, mostly RBCs.
	2:45	5 or 6 amoebae with from 2–5 ingested leucocytes.		Slide treated with Wright's stain showed 3 amoebae with ingested WBCs.
	3:20	Several amoebae with a few ingested bodies, mainly leucocytes.		
		Slide stained with iron-haematoxylin showed 5 or 6 amoebae with ingested leucocytes.		

[*] RBC=Red blood corpuscle. [†] WBC=White blood corpuscle.

remains were found within the organisms. The actual ingestion of the different cells was never noticed although an interesting observation in respect to one manner of engulfment was made on a slide of No. 38.

An amoeba was moving around and over toward a large degenerate leucocyte. A long pseudopodium was then sent out, which, flowing over the white cell, was soon pulled back toward the amoeba in such a manner as though to scrape out the interior of the cell. Two long, granular strands were dragged out from the leucocyte as the organism moved away, leaving a slender connection which, gradually diminishing, soon snapped apart. The amoeba seemed to have taken in some of the granules but, as the organism was already crowded with ingested material, it was difficult to know how much of the leucocyte was actually ingested.

The stained slides made from these preparations amply substantiated the observations made on the living material, since many amoebae were found with ingested cells that were typical of either the white or the red blood corpuscles of the surrounding medium. The iron-haematoxylin stain accentuates the nuclear material of the polymorphonuclear cell, coloring it a very dark blue. The appearance of the nucleus with this stain is quite different from the appearance given in the dried smears of the routine blood stains and shows three or four small round bodies closely packed together like a clover leaf toward one side of the cell. Many clumps of leucocytes stained in this fashion were noticed on the slides, while the same bodies either intact or in various stages of disintegration were also found within the amoebae. A comparison of figures 8 and 12 on plate 17 will emphasize this point more clearly. The erythrocytes were usually colored intensely by this stain but their ovoid shape, biconcavity, and often crenated appearance were clear diagnostic evidence, so that it was not difficult to recognize them when engulfed by the amoebae (pl. 17, fig. 6).

Wright's stain, on the other hand, fails to bring out the nuclear features but accentuates the granulations of the polymorphonuclear cells, which in the guinea pig are colored a brighter pink than are those in man, being more like the human eosinophiles. The cytoplasm of the amoebae is stained a light blue while the nucleus is either imperceptible or else shows as a darker, bluish ring, as may be seen in figure 2, plate 16. The presence of the ingested leucocytes with their bright pink cytoplasmic granules and very light blue nucleus

TABLE 4

Culture of E. gingivalis Treated with Washed, Guinea Pig Peritoneal Cells

	Time	Culture No. 38				Culture No. 44			
		Number of amoebae	Observations			Number of amoebae	Observations		
			Fresh preparation	Wright's stain slide	Iron haematoxylin slide		Fresh preparation	Wright's stain slide	Iron haematoxylin slide
RBC not hemolysed	24 hours	920	40 with ingested cells mostly RBC.	Many amoebae, 4 or 5 with ingested RBC.	Many amoebae, none with included cells.	75	22 with ingested RBC, some with 8 or 9.		4 or 5 amoebae with RBC. One with a WBC.
	48 hours					105	35 with ingested RBC. One with WBC.	8 amoebae with RBC.	15 amoebae with RBCs.
RBC hemolysed	24 hours	507	45 with ingested cells, few WBC and some RBC remains.	2 amoebae with undissolved, RBC, one with WBC.	One amoeba with WBC, 2 with RBC. remains.	76	20 with included cells, largely RBCs.		Many amoebae but no ingested cells.
	48 hours					200	Many with RBCs.	3 amoebae RBCs, one with WBC.	
L.E.A. Controls	24 hours	480	Many with shiny bodies.			58	6 or 7 with shiny bodies.		

is thus clearly accentuated against the uniform, bluish background of the organism (pl. 16, fig. 1). The pink-tinged erythrocytes are also clearly visible when present in an amoeba (pl. 16, fig. 4).

Table 4 gives the results of adding a certain number of drops of exudate from both tubes to cultures of No. 38 and No. 44 in L.E.A. medium. Observations were made in 24 and 48 hours. Excellent growth was obtained in the enriched cultures and many organisms were found with ingested cells, the erythrocytes being especially favored. Not all of the red blood corpuscles were completely hemolyzed in the exudate from tube 1, so that many of these partially disintegrated cells were found within the amoebae. Stained slides were also made, confirming the findings in the fresh preparations.

It seems quite evident from the results of both the direct microscopic and the test tube observations, that *E. gingivalis* is capable of ingesting *in vitro* both red and white washed guinea pig blood cells with a slight preference for the former when both are present. However, it does not subsist on these cellular substances to the exclusion of the bacteria, since many bacteria were to be found in the amoebae coincident with the included cells (pl. 16, fig. 1.) Neither Kofoid and Swezy (1924) nor Child (1925) found ingestion of erythrocytes by *E. gingivalis* in direct smears from the mouth, although the latter worker clearly demonstrated the presence of ingested leucocytes by this amoeba and shed light on the process of ingestion.

(*d*) Effect of Gastric and Duodenal Fluids and of Bile upon *Endamoeba gingivalis in vitro*

Although Kofoid and Swezy (1924) have clearly shown that there are distinct morphological differences between *E. gingivalis* and *E. dysenteriae*, the amoeba of the bowel, yet there is enough similarity in their amoeboid forms to cause slight uncertainty in diagnosis to those unfamiliar with the finer points of differentiation, in case the former organism should withstand the gastric and intestinal fluids and be subsequently recovered in the faeces. For this reason it seemed of interest to determine what effect gastric and intestinal fluids would have upon *E. gingivalis in vitro.*

The materials were obtained both from patients in the Infirmary at the University of California, through the courtesy of the technician, Miss Eleanor Godfrey, and from several private patients, through the courtesy of Dr. L. M. Boyers. Whenever possible the degrees of free hydrochloric acid and of total acidity of the gastric juice were determined by the usual methods. The fluids were used as soon as possible after collection in order to prevent loss of activity. Active cultures of *E. gingivalis* were employed, either No. 13 or No. 44, both being from the person having a slight case of gingivitis. Observations were made on the behavior of *E. gingivalis* both when a drop of fluid was run beneath the cover slip of a slide preparation and when the amoebae were previously mixed with the fluid in a test tube. In the latter case the fluid was used both undiluted and in dilutions of 1–1 and 1–10 in physiological NaCl solution. Control tubes of amoebae and saline were also prepared.

TABLE 5

TEST TUBE EXPERIMENTS WITH E. GINGIVALIS AND HUMAN GASTRIC JUICE

Fluid	Free HCl	Total acidity	Time duration	Number of amoebae in the different dilutions of gastric juice			
				Undiluted	1-1 dilution	1-10 dilution	control
No. 1	4	11	1 hr.	6—active	8—active	3—active	5—active
			2 hrs.	no amoebae	7—active	1—active	
			3 hrs.	no amoebae	2—active	1—sluggish	no amoebae
			4 hrs.	no amoebae	18—active	no amoebae	no amoebae
			5 hrs.	no amoebae	32—active	no amoebae	
No. 2	5	33	1 hr.	no amoebae	no amoebae	2—rounded up	4—active
			2 hrs.	no amoebae	no amoebae	no amoebae	few degenerate
No. 3	34	65	1 hr.	no amoebae	no amoebae	no amoebae	20—active
			2 hrs.	no amoebae	no amoebae	no amoebae	65—active
No. 6	3	17	1 hr.	1—thickened up	3—one active, two sluggish	3—sluggish	12—active
			2 hrs.	no amoebae	1—sluggish	8—degenerate	1—slightly motile
			3 hrs.	no amoebae	no amoebae	no amoebae	5 or 6 rounded up, non-motile

Table 5 gives the results of the test tube experiments when human gastric juice was mixed with a few drops of material from a culture of *E. gingivalis*. Hourly observations were usually made until the amoebae had disappeared. Fluids No. 1 and No. 2 were obtained from the same person, but on different occasions, the first one being removed five hours after breakfast and showing a very low acidity, while the other one was obtained one and a half hours after breakfast. The free HCl was again low but the total acidity had increased. Fluids No. 3 and No. 6 were from two different persons, the latter having low acid values and the former having a normal amount of free HCl and of total acid.

The fluids which had the lowest degree of acidity (No. 1 and No. 6) permitted the amoebae to exist for at least one hour in the undiluted gastric juice and for a longer time in the 1–1 and 1–10 dilutions (even for five hours in the case of No. 1). The fluid with the normal amount of acid, on the other hand, evidently destroyed the organisms immediately in all dilutions, while the control contained many active amoebae.

TABLE 6

SLIDE OBSERVATIONS UPON E. GINGIVALIS WITH HUMAN GASTRIC JUICE

Fluid	Free HCl	Total acidity	Length of time on slide	Observations
No. 1	4	11	7 min.	Amoeba active, with broad blunt pseudopodia.
			15 min.	Amoeba sluggish.
			18 min.	Still moving.
			20 min.	Active.
			30 min.	Active.
No. 2	5	33	1 min.	Watched one amoeba thicken up, send out short, explosive pseudopodia.
			2 min.	Pseudopodia extended, only slight movement to organism.
			3 min.	Amoeba quite motionless.
			5 min.	Amoeba thickened up, apparently dying.
No. 3	34	65	2 min.	Watched an amoeba round into a ball, sending out short, knob-like pseudopodia.
			4 min.	Thickened up and became completely round with a granular, faded appearance.
			5 min.	Amoeba gradually faded out, the nucleus being the last to disappear.
			6 min.	Just about gone from sight.
No. 4	Neg.	40	1 min.	Watched an amoeba round up with knobs over the surface.
			2 min.	Slightly active, though thickened up with short pseudopods.
			8 min.	Pseudopodia more rounded and normal.
			13 min.	Amoeba became rounded up again with knobs.
			18 min.	Slightly motile.
			21 min.	Amoeba became quite rounded up, only very slight projections.
			22 min.	Beginning to degenerate.
			25 min.	Amoeba degenerating.
No. 5	3	50	1 min.	An amoeba rounded up, extending knob-like pseudopodia.
			3 min.	Same amoeba became fairly active.
			8 min.	Slight motility, with spread out pseudopodia.
			11 min.	Still motile, though very granular. Many knobby pseudopodia.
			17 min.	Still motile.
			24 min.	Slightly motile.
			29 min.	Amoeba more rounded up, one or two small knobs.
			34 min.	Motile, granular pseudopodium.
			35 min.	Amoeba became smaller, granular, but with slight motion.
			60 min.	Amoeba rounded up, disintegrating.
No. 6	8	17	1 min.	Amoeba shrank slightly, pseudopodia pulled in.
			2 min.	Became more shrunken, then shot out a pseudopod.

These findings were confirmed by the microscopic slide observations, using the same fluids and two others in addition (No. 4 and No. 5), both of which were negative for free HCl although normal for the total acidity. When the HCl was high the amoebae were destroyed within five or six minutes after the first contact with the undiluted gastric juice, but remained alive from sixteen minutes to one hour when it was reduced in amount or lacking entirely. The detailed notes for these observations are given in table 6.

From all indications it seems that should *E. gingivalis* be swallowed with the saliva it might survive passage through the stomach only in case of either a great reduction or of a total absence of free HCl. Under normal circumstances it would soon be destroyed by the gastric fluid.

Even though the amoebae survive this first passage, they might be destroyed by the digestive juices in the duodenum, since the organisms were without the resistant outer wall characteristic of the cysts of the intestinal amoebae, which enter the bowel by the food route in the encysted stage. To determine that point, fractionated, duodenal contents were obtained from several patients of Dr. L. M. Boyers, the fraction having much bile being received into a separate container from the earlier portions recovered by duodenal drainage. Direct slide observations were made upon the behavior of *E. gingivalis,* both with the first portion of duodenal fluid containing little if any bile, and with the later portion composed of nearly pure bile.

The amoebae appeared to withstand contact with the duodenal contents for about five or six minutes, after which they became shrunken and degenerate. Behavior in the presence of the concentrated bile was quite characteristic, occurring several times in the same manner with material from three different people. Details are given in table 7. The organism was immediately affected by contact with the bile, shrinking up rapidly, and then suddenly exploding like a bombshell, leaving nothing behind except tiny granules. These soon scattered into the surrounding fluid and with them went all visible signs of the amoeba.

This explosive behavior of *E. gingivalis* when in contact with human bile seemed to indicate a sudden change from an isotonic to a strongly hypertonic solution probably caused by an increase in the specific gravity of the medium. Since the patients from whom the material was collected were often given a dose of a saturated solution of $MgSO_4$ in order to procure a free flow of bile into the duodenum,

TABLE 7

SLIDE OBSERVATIONS UPON E. GINGIVALIS WITH DUODENAL CONTENTS HAVING A VERY SMALL AMOUNT OF BILE

Fluid	Length of time on slide	Observations
No. 7	1 min.	Amoeba shrank up after adding the fluid, became much smaller.
	2 min.	Rounded up, very small.
	3 min.	Tiny points appeared over the organism which became still smaller.
	4 min.	Amoeba very small and degenerate.
No. 8	1 min.	Amoeba protruded pseudopods.
	2 min.	Rounded up.
	3 min.	Amoeba degenerating, much shrunken.
	6 min.	Very much shrunken, dead.

DUODENAL CONTENTS MIXED WITH MUCH BILE

Fluid	Length of time on slide	Observations
No. 6		Amoeba was seen to explode immediately after addition of bile to the slide.
No. 9	1 min.	Amoeba thickened up when the fluid first ran over it. Showed slight projections.
	2 min.	Shrank up rapidly, pseudopodia drawn in.
	3 min	Amoeba very shrunken. Suddenly the whole organism exploded into very small pieces, only granules being left.
		Watched another amoeba explode in the same manner after 4 minutes exposure to the bile
No. 10	1 min.	Amoeba active with many pseudopodia.
	2 min.	Rounded up, pseudopodia withdrawn.
	3 min.	Amoeba quite round, beginning to fade out.
	4 min.	The nucleus suddenly blew out, the cytoplasm was all faded away, nothing left but an empty shell.
		Watched another organism explode in 2 minutes.

EFFECT OF ANIMAL BILE ON E. GINGIVALIS

Fluid	Length of time on slide	Observations
Guinea pig		Bile added to the slide did not explode the amoebae.
	4 min.	Amoeba rounded up with knobs.
	12 min.	Nearly all the amoebae had disappeared from the slide.
Cat	1 min.	Amoeba motile.
	3 min.	Still motile.
	7 min.	Amoeba disappeared.
		Watched another amoeba in a more dilute part of the slide.
	1 min.	Slightly motile.
	10 min.	Still slightly motile, elongated.
	20 min.	Amoeba sluggish.
	30 min.	Slightly motile.
	40 min.	Rounded up.
	1 hr.	Amoeba still showing very slight motion.

EFFECT OF SATURATED $MgSO_4$ SOLUTION ON E. GINGIVALIS

Fluid	Length of time on slide	Observations
	1 min.	Amoeba shrunken up.
	4 min.	Very shrunken and degenerate—Did not explode.
		Watched another amoeba after addition of more $MgSO_4$.
	1 min.	Waves of pseudopodia appeared over the organism.
	2 min.	Amoeba rounded up with knobs all over.
	3 min.	Very shrunken, apparently dead.

there was a possibility that this substance, rather than the bile alone, might have caused the explosion of the organisms. The salt was not introduced into the duodenal tube until after the first flow of liquid had been collected, so that it would not be present in the earlier fractions. It was also rinsed from the tube after the dose was given. For this reason there should not have been a very strong concentration in the fluid later collected.

To determine the effect of saturated $MgSO_4$ upon the amoebae, a drop of the salt solution was added to the slide preparation of *E. gingivalis*. The organism did not explode, but shrank up, gradually degenerating in three or four minutes. Evidently this substance was not the direct cause of the former explosions.

Bile obtained directly from the gall bladder of both a guinea pig and a cat was then placed in contact with the amoebae. In neither case did the organisms explode. The guinea pig bile caused the amoebae to round up and gradually disappear in about eight minutes. Concentrated cat bile seemed to have about the same effect. However, one organism was watched for about an hour in a more dilute portion of the slide and seemed to remain viable and sluggishly motile during this period.

It appears from these several results as if whatever caused the explosive disappearance of *E. gingivalis* in the earlier observations was a peculiarity of human bile after being withdrawn from the patient. Perhaps a combination of medicinal substances, such as the $MgSO_4$, with the salts from the bile produced a highly concentrated solution that was hypertonic to the amoebae.

From the evidence given *in vitro* it seems probable that the mouth amoebae may be able to pass through the stomach in cases where the free HCl is absent or much reduced, but that they would very likely be destroyed in the duodenum, especially at the time of active bile secretion. In case the bile were absent or reduced they might possibly be found in the lower bowel. The chances of finding *E. gingivalis* in the faeces, however, seem very slight.

SUMMARY

1. *Endamoeba gingivalis* will thrive in the presence of filtered human saliva, provided a solid substrate of coagulated egg is present, and will also grow in Locke-albumen medium having a base of coagulated serum or of agar if the latter has not too slippery a surface.

2. Both washed red blood corpuscles and leucocytes from the guinea pig were found ingested by *E. gingivalis,* evidence being obtained from observations on living material and from stained slides. When both types of cells were present, the preference was for the erythrocytes.

3. *E. gingivalis in vitro* is unable to withstand contact with human gastric juice containing the normal amount of acid, but will survive for several hours in fluid lacking, or with a reduced amount of free HCl. The organisms rapidly explode in the presence of human bile but merely shrink up and gradually degenerate when in contact with concentrated bile from both the cat and the guinea pig. Saturated $MgSO_4$ solution also produces a shrinkage and gradual disappearance of the amoebae, but not the characteristic explosive disappearance given by contact with human bile *in vitro.*

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EXPLANATION OF PLATES

PLATE 16

All figures are drawn and colored from culture material of *E. gingivalis* stained by Wright's method. × 2500.

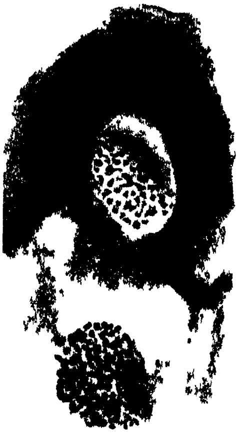
Fig. 1. An amoeba from an L.E.A. culture of *E. gingivalis* to which washed guinea pig peritoneal cells were added. Two ingested polymorphonuclear leucocytes and several groups of bacteria may be seen.

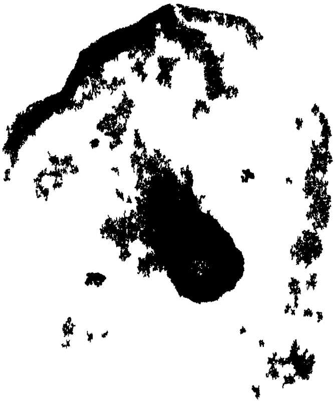
Fig. 2. An amoeba from the same culture showing the blue ringed nucleus and a few granular leucocytic remains (stained red).

Fig. 3. Two polymorphonuclear leucocytes from the peritoneal exudate of the guinea pig, surrounded by bacteria. Note the same morphological appearance and staining reactions as the cells found in the amoebae.

Fig. 4. An amoeba with an ingested guinea pig erythrocyte.

Fig. 5. An amoeba with many vacuoles and three ingested leucocytes.





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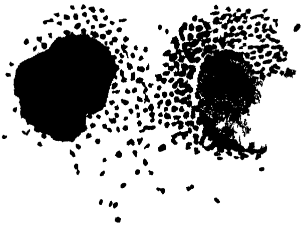

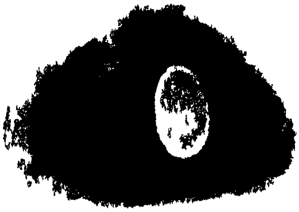

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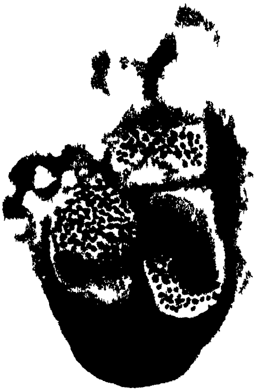

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PLATE 17

All figures are drawn from cultural material of *E. gingivalis* after fixation in hot Schaudinn's fluid and staining in iron haematoxylin. × 2500.

Fig. 6. An amoeba from an L.E.A. culture of strain No. 44 to which washed guinea pig peritoneal cells were added. An ingested, slightly crenated, deeply stained erythrocyte may be seen in the endoplasm.

Fig. 7. An amoeba from a culture of strain No. 13 showing the elongated form and characteristic nucleus.

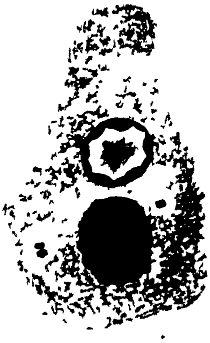
Fig. 8. An amoeba from a culture of strain No. 38 showing the nucleus, many bacteria, and an ingested leucocyte.

Fig. 9. A small amoeba from an L.E.A. culture of strain No. 13.

Fig. 10. An amoeba from a culture of strain No. 13.

Fig. 11. An amoeba from a culture of strain No. 44 to which washed, unhemolyzed, guinea pig peritoneal cells had been added. Note the ingested leucocyte and also the crenated erythrocyte close to one side of the organism.

Fig. 12. Two guinea pig leucocytes found exterior to the amoebae in the surrounding medium. The nuclei have the same appearance as those of the cells found within the amoebae.



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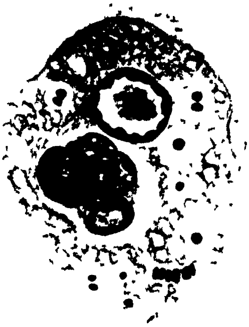

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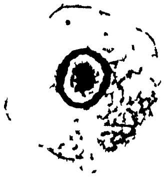

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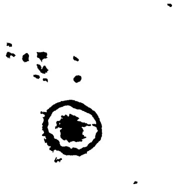

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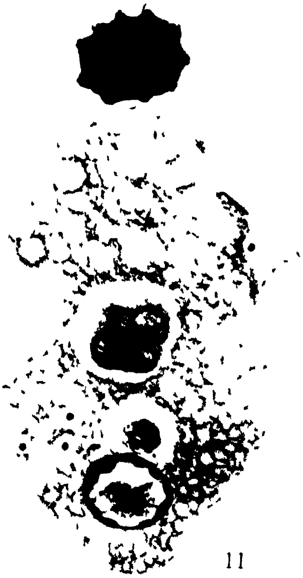

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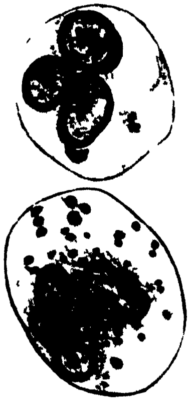

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ON OXYPHYSIS OXYTOXOIDES GEN. NOV., SP. NOV.

A DINOPHYSOID DINOFLAGELLATE CONVERGENT TOWARD THE PERIDINIOID TYPE

BY

CHARLES A. KOFOID

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The typical Dinoflagellata (Diniferidea) with the girdle in which lies the transverse flagellum, fall into two groups, those without skeleton (Gymnodinioidae) and those with an exoskeleton composed of discrete plates. The latter include two quite distinct tribes, the Dinophysoidae, with a vertical fission plane dividing the skeleton at binary fission into right and left halves, and the Peridinioidae, in which the fission plane is oblique, passing from the right-anterior region obliquely to the left-posterior one along suture lines between the discrete plates.

An additional difference of basic significance between these two thecate groups lies in the skeletal patterns of the two. The Dinophysoidae are horizontally divided into three zones or segments only, namely, the epitheca, the girdle, and the hypotheca, each consisting of a right and a left moiety. These horizontally arranged parts in the dinophysoid group are much more difficult to separate than are the plates of the peridinioid group. The right and left valves part readily in the dinophysoid theca, the epithecal segment separates from the girdle, but the hypothecal and cingular segments, while morphologically distinct, are separable with difficulty.

In the peridinioid dinoflagellates, the exoskeleton is parted into five instead of three horizontal segments, both the epitheca and the hypotheca being subdivided into two zones, the apical and precingular, and the postcingular and antapical, respectively, while the girdle remains as a single belt only, as in the dinophysoid group.

Not only is the peridinioid skeleton subdivided horizontally; it is also subdivided by vertical sutures which take on, in most genera, at, or near, their junctions with the horizontal sutures, the angular features of the perimeter of a polygon, the apical plates fitting alternately between the precingulars, and the postcingulars being likewise adjusted to the antapicals. This increase of the number of sutural lines, both horizontal and vertical, results in an increase in the number of plates. Whereas in the Dinophysoidae there are uniformly only six plates, in the Peridinioidae there may be as many as twenty, or even more. Thus *Ceratium* has in the five horizontal series, excluding the region of the ventral plate, 4, 5, 5, 5, and 1 plate, respectively. In *Oxytoxum* the corresponding formula is probably 5, 5, 5, 5, 1.

These marked differences in the patterning of the subdivisions of these two types of thecate dinoflagellates are accompanied by marked differences in form and in the organs of flotation. In the Dinophysoidae there is a tendency toward bilateral symmetry, bilateral compression, absence of processes of the body proper (except in *Dinophysis*, *Triposolenia*, and *Amphisolenia*), and toward the excessive development of the lists of the girdle and sulcus into fins and sails.

In the Peridinioidae, on the other hand, there is little if any bilateral tendency, or bilateral compression, but rather tendencies to sphericity, to dorsoventral compression, and to the extension of the body in single anterior and two asymmetrical posterior prolongations or horns, which function as organs of flotation, as in *Ceratium*. The lists of the girdle and sulcus remain undeveloped, except in *Ceratocorys*.

A third marked difference between these two groups is seen in the fact that the epitheca of the Dinophysoidae is very slightly developed. In consequence, the girdle is located anteriorly in all the genera and most of the species of this group. In *Phalacroma* only does it attain anything like the development or relative proportions which prevail in the Peridinoidae.

As a result of these marked differences in the evolution of the skeleton in these two groups of thecate dinoflagellates, their separation, in all their varied forms, is at once feasible without possibility of confusion. There is, as a general rule, no doubt as to the relationship of any dinoflagellate of these two groups. The proportions of epitheca and hypotheca and girdle and sulcus at once allocate the species in question to one or the other group. There has been one exception, however, as when Entz (1902) linked *Ceratocorys,* a genus of the Peridinioidae, with *Phalacroma,* a genus of the Dinophysoidae, because of the convergence of *Ceratocorys* in the development of its cingular lists and its left sulcal list, and in the reduction in the development of its epitheca, toward *Phalacroma.*

It is the purpose of this paper to invite attention to another instance, of the most striking nature, of convergence between genera of the two tribes, Dinophysoidae and Peridinioidae. In this instance, however, the convergence is in a direction the reverse of that noted by Entz (1902). In his instance a peridinioid genus resembled a dinophysoid. In ours a dinophysoid genus has taken on the facies of a peridinioid genus.

Our material consists of a few specimens of a very unusual species of a new genus of Dinoflagellata from two localities. The genus was first detected by us in a plankton collection made by the U.S.S. *Albatross* of the U.S. Bureau of Fisheries at Loring, Alaska, September 15, 1905. This paper is published by the permission of the United States Commissioner of Fisheries.

One additional specimen only was later detected in a plankton collection made by the San Diego Marine Biological Station (the predecessor of the Scripps Institution of Biological Research) on May 29, 1904, in the San Pedro Channel. The haul was made at the surface with a No. 20 silk net. The catch contained a plankton of distinctly northern facies, with many of the same species of *Ceratium*, *Peridinium*, etc., found also in the plankton from Loring, and other Alaskan localities. The vernal plankton of the San Diego region exhibits each year at this season the influx of northern species.

Oxyphysis gen. nov.

Plate 18, figures 1–4

General diagnosis.—Body with the facies of *Oxytoxum* but the fission plane and plate formula (2–2–2) of the Dinophysoidae. Epitheca well developed, sulcus short, sulcal lists feebly developed, girdle impressed, not anterior, fission rib near the girdle. Eupelagic.

Oxyphysis oxytoxoides sp. nov.

Description.—Body *Oxytoxum*-like, resembling *O. challengeroides* Kofoid in size, proportions, location of girdle, cingular lists, small sulcus, sulcal lists, apex, and antapex.

Body is asymmetrically and unequally fusiform, slightly compressed laterally, and acute (in lateral view) at both ends. Length five times the dorsoventral diameter in the girdle and seven times the transdiameter in the same region. It is widest (transdiameter) at 0.5 girdle width behind the girdle and thickest (dorsoventral) a little farther posterior at the level of the posterior end of the sulcus, a little behind the middle of the body.

Epitheca an asymmetrical cone with unequally concave sides, asymmetrical apex, and flaring base. Base with more dorsal than ventral or lateral flare (fig. 2), and a little more of a shoulder on the right (fig. 4) than on the left side. Apex contracts abruptly to a blunt end (in ventral view) which is extended on one valve only in a short, acute blade 0.20 to 0.33 girdle width in length. This is found on either the right or the left side of the suture, not on both (see figs. 3 and 4), and may belong to the older half of the skeleton and be the result of regulatory adjustments at fission. In support of this view note presence of a similar blade at the antapex in figure 4 on the *same* side as the anterior blade, though this posterior blade is not present in a second individual (figs. 2, 3) which had a smaller anterior blade. Difference in ventral contour in the two individuals (see figs. 1 and 2) due in part to angle of observation and also to the fact that second specimen (fig. 2) is megacytic, and regulatory resorbtion may be in progress posteriorly. Epitheca has a height or length of a little less than one-third the whole body or 1.75 dorsoventral diameter in the girdle. Base has dorsoventral and transdiameter of about 0.66 and 0.60, respectively, of its altitude.

Girdle is 0.4 of the total length from the anterior end, and about 0.11 of the total length in width. It forms a descending right spiral of a single turn with a distal displacement of scarcely 0.16 girdle width. In most of the Dinophysoidae there is no such displacement, the girdle lying in one plane. This displacement is similar in type and degree to that in some species of *Oxytoxum* (compare figs. 3 and 4 with 5 and 6). Furrow rather deeply impressed into the body forming a subsemicircular trough in cross-section, somewhat deeper dorsally and ventrally than laterally (compare figs. 1 and 2 with 3 and 4).

Cingular lists subequal, anterior a trifle narrower than posterior. Their width is from 0.25 to 0.50 girdle width. Both inclined anteriorly as is the case rather generally among the Dinophysoidae, though the inclination is not very steep. They are true lists, being thin, hyaline membranes and not extensions of the body or a mere heavy ridge as are the margins of the girdle in *Oxytoxum* (see figs. 5–8). They are devoid of ribs, which are often found in the wider cingular lists of other genera of the Dinophysoidae. They spring from heavy basal ridges, the anterior one running out upon the epitheca on the right valve at the junction of the two ends of the girdle (fig. 4), and the posterior one, being continuous with the ridge, encircling the sulcus and forming the basal line for the right and left sulcal lists. The two are connected by cross-ribs of similar structure across the furrow on either side of the midventral suture, and probably also by the sides of the middorsal suture (fig. 1), though not so in a megacytic individual with a wide dorsal intercalary zone (fig. 3).

Hypotheca is about 0.55 of total length of body in length, or about 3 dorsoventral diameters in the girdle. Transdiameter greatest 0.5 girdle width behind the girdle. This transdiameter is a trifle over 2 girdle widths in the non-megacytic phase (fig. 4). Dorsoventral diameter greatest near or a little posterior to end of sulcus and is 3.0 to 3.5 girdle widths. Hypotheca contracts uniformly posteriorly on both sides (fig. 4) to an obtuse, obliquely truncate antapex. In lateral view it has an elongated, ovate contour with a more accentuated contraction two thirds of its length from the girdle, contracting distally from that level with subconical contour and rounded antapex. In some individuals one of the valves, the right in figure 4, bears an acute, thin blade about 0.3 girdle width in length. The antapical end has a noticeable ventral deflection as in some species of *Oxytoxum* (compare fig. 1 with 7 and 5).

Sulcal lists feebly developed. They are thin, hyaline sheets, rising from a basal ridge continuous anteriorly with that of the postcingular list. Right and left sulcal lists also continuous with decurrent distal and proximal ends respectively of postcingular list. Right list (figs. 1 and 4) minute, and only half the length of the left and about 0.5 girdle width in length. Left list a little more than a girdle width in length, decurrent distally, and curves over to the right distally; crosses suture very close to girdle. Both lists devoid of structural differentiations other than the two ribs. There is a slight development of the anterior sulcus upon the epitheca in the indentation and anteriorly directed ridge (pl. 18, fig. 4) arising from the anterior cingular ridge in the midventral region. This sulcal extension is even more highly developed in *Pseudophalacroma nasutum*.

Fission rib (fig. 4) located at this crossing immediately behind girdle and is double. There is another rib, the posterior one (fig. 2), near posterior end of left list.

Fission suture a zigzag sagittal line (figs. 3 and 4) along which the theca parts at binary fission (fig. 4). Line formed by interdigitation of minute teeth of the opposed edges. Bordered on either side (figs. 3 and 4) by parasagittal lines which mark off intercalary zone. As these cross girdle on the ventral face, they expand into the heavy ridges noted above, and presumably form similar ridges across girdle on the dorsal side. There is an abrupt jog in suture line just anterior to flagellar pore (fig. 4), in which it passes obliquely posteriorly, parting the two fission ribs and carrying the fission line posteriorly entirely to the left of sulcus.

Sulcus the indented area between the two sulcal lists. Contains elongated flagellar pore located immediately behind girdle. Sulcus may be regarded as including also the area between the two ventral cross-ribs in the girdle. Anteriorly it slightly indents epitheca but does not extend as far anteriorly upon it as does the sulcus or "longitudinal furrow" of *Oxytoxum compressum* (*l. f.*, fig. 7).

Thecal wall differentiated in small, subequal, uniform, subpolygonal reticulations over whole surface (fig. 1) except the two ends and near suture line toward which reticulum fades out in free ends. There are fourteen such polygons in first row behind postcingular list, all porulate. Elsewhere only scattered pores occur, rather evenly spaced, in some of the polygons. Reticulum extends only halfway to top of epitheca and three-fourths of distance to antapex. In these restrictions of the reticulations it resembles some species of *Oxytoxum*, e.g., *O. sceptrum* Stein. Thecal wall in furrow of girdle (fig. 1) has a few scattered incomplete reticulations, partial vertical riblets, and irregularly placed pores.

There is also a *ventral pore* located on right parasagittal line bordering ventral suture about one-third of height of epitheca from apex. A similar pore in a comparable location is to be found in certain of the Peridinioidae, such as *Oxytoxum gigas* Kofoid (1907, pl. 10, fig. 59), in *Heterodinium* (loc. cit., 1907, pl. 8, figs. 47–51), and *Centrodinium* (*loc. cit.*, pl. 9, fig. 53). This pore is not a structural characteristic of the Dinophysoidae.

Dimensions: Length, 63 to 68μ; transdiameter on posterior cingular list, 17μ; in girdle, 10μ; on widest part of hypotheca, 15μ; dorsoventral diameter on postcingular list, 21μ; in girdle, 13μ.

Thecal wall of *Oxyphysis* consists of two epithecal, two cingular, and two hypothecal plates, as in all the Dinophysoidae. Sagittal suture parts right from left plate in each of the three zones. The genus *Oxytoxum*, on the other hand, has the five horizontal zones of plates of the Peridinioidae with 5, 5, 5, 5, and 1 plate in the respective zones, with the possibility of some changes in certain species in the apices.

Cell contents of this species little known, as the cell has been seen only in material preserved in formalin. The nucleus(?) appears to be the larger oval body at the level of the postcingular list (fig. 4). In one megacytic individual (fig. 4) the cytoplasm was crowded with spheroidal bodies of uniform size but unknown nature, apparently not nuclei.

The *megacytic stage* is marked by the expansion of the intercalary zones, which are transversed by fine, close-set, parallel, oblique striae (fig. 4). This zone is widest posteriorly, and wider dorsally than ventrally. In the normal phase (fig. 3) this zone is very narrow and of uniform width along the sides of the suture line. In the individual seen by us the thecal wall was splitting along the suture, most widely in the posterodorsal region.

DISCUSSION

The genus *Oxyphysis* thus presents the challenging phenomenon of the convergence of a genus of the Dinophysoidae toward the facies of the genus *Oxytoxum* of the Peridinioidae. The two tribes of thecate girdle-bearing Dinoflagellata are thus strikingly united in the genus *Oxyphysis,* which combines in one unit the basic skeletal pattern of the Dinophysoidae with contour and proportions of a genus of the Peridinioidae. There are no other known genera of the former tribe which exhibit a comparable resemblance. The two plate patterns are respectively fundamentally characteristic of the two tribes.

The details of the convergence of *Oxyphysis* toward *Oxytoxum* can be seen on a comparison of our new species, *O. oxytoxoides,* with several species of *Oxytoxum.* For this purpose we have reproduced (see Kofoid, 1907, pl. 10, figs. 62, 65, 63, and 61) the outline figures of four species, to wit, *O. subulatum, O. challengeroides, O. compressum,* and *O. curvicaudatum* (figs. 5–8), which display the range in form within that genus from the subspheroidal, as in *O. curvicaudatum* (fig. 8), to the elongated form, as in *O. subulatum* and *O. challengeroides* (figs. 5 and 6). It is to the elongated species that *Oxyphysis* most nearly converges, especially to *O. challengeroides* (fig. 6).

The structural features in which *Oxyphysis oxytoxoides* approaches *Oxytoxum challengeroides* are the following: (1) size, the length and transdiameter of the two being respectively 68 and 15, and 80 and 22 microns; (2) shape and proportions of the epitheca, even to the greater extension of the dorsal shoulder as in *O. subulatum* (fig. 5); (3) the location of the girdle at the anterior third of the total length; (4) its deep indentation into the body wall; (5) the slight posterior displacement of its distal end; (6) the shape and proportions of the hypotheca; (7) the ventral deflection of the antapex as in *O. compressum* and *O. curvicaudatum* (figs. 7 and 8); (8) the pointed (in lateral view only) and asymmetrical apices; and (9) the feeble development of the cingular and sulcal lists.

The significance of these resemblances are the more striking in the light of the following comments on the items enumerated, to wit: (2) No genus of the Dinophysoidae has an elongated pointed epitheca. (3) With the exception of a few of the more primitive and simple species of *Phalacroma,* all of the Dinophysoidae have the girdle at or near the anterior end of the body. (4) The girdle is never indented into the body wall as a deep trough in any of the Dinophysoidae, and (5) it forms a flat ring, not a descending spiral with a distal displacement. (6) The hypotheca of the Dinophysoidae is rounded or truncate and but rarely tapering and pointed, and (7) never ventrally deflected. (8) Pointed apices are practically unknown in the Dinophysoidae. (9) Cingular and sulcal lists are more highly developed in the Dinophysoidae than elsewhere in the Dinoflagellata. The structural features which thus dominate the facies of *Oxyphysis* are those of *Oxytoxum* and not those of any known Dinophysoidae.

The convergence of these two genera, *Oxyphysis* and *Oxytoxum,* is all the more challenging in view of the respective positions of these two genera in their own tribes. Neither of them is a primitive genus. Both are unquestionably to be placed rather high up among the diverging genera of their respective tribes. They are both specialized rather than primitive genera, distal rather than proximal twigs in the two diverging branches of the thecate Dinoflagellata (Diniferidea).

The primitive simple genera in both branches have as their simplest and presumably most primitive species those which are (1) relatively small, (2) generally spherical or nearly so, (3) with the girdle subequatorial, not impressed and not spiraled, and (4) with lists having little or no differentiation.

These two genera both exhibit their evolutionary advance to a relatively high position in their respective tribes by (1) the elongation of the body, (2) differentiated asymmetrical apices, (3) specialized epitheca, (4) indented, slightly spiraled girdle, and (5) reticulated or ribbed thecal wall.

The genus *Oxytoxum* contains 26 species which exhibit a wide range of specific diversification, mainly in the locations of the girdle, differentiations of the apex and antapex, in the spiraled course of the girdle, and in the finer structure of the thecal wall, which is variously reticulated and ribbed (figs. 5–8). The genus is represented in all seas from cold temperate to tropical latitudes. Ordinarily its species are not very abundant in collections made with plankton nets of even the finest silk. Lohmann's (1902) results with the centrifuge and our examinations of the contents of *Salpa* stomachs indicate that some at least of its numerous species are abundantly represented in the marine plankton both of coastal waters and the high seas. It appears to be a dominant and successful genus whose species are well adapted to the pelagic life.

In converging toward *Oxytoxum,* the genus *Oxyphysis* has assumed the habitus and facies of at least a fairly successful genus of the Peridinioidae. However, it is a member of the Dinophysoidae, a tribe also including very successful components of the marine plankton. Moreover, it is more clearly related to the genera *Dinophysis* and *Phalacroma,* both of which contain the most successful and abundant species of the tribe, than to the bizarre, relatively rare, and more highly specialized genera such as *Triposolenia* and *Histioneis.* Its genetic affiliations in the evolutionary stock from which it sprang are with the successful and well adapted members of its tribe. In addition, it lives in precisely the same habitat with a number of species of *Oxytoxum, Dinophysis,* and *Phalacroma.* Its convergence from the facies of the Dinophysoidae to that of the Peridinioidae has not seemingly made for it an improvement in its adaptive provisions for pelagic life, judging by the speciation, relative abundance of individuals, and present geographic distribution of the species of *Oxytoxum* on the one hand and of *Dinophysis* and *Phalacroma* on the other. In either garb it seems that it might thrive equally well and in the same habitat.

Observation seems not to reveal any marked differences in behavior in flotation and locomotion between *Oxytoxum* on the one hand and such dinophysid genera as *Phalacroma* and *Dinophysis* on the other which might afford a basis for the convergence of *Oxyphysis* toward *Oxytoxum* by reason of the assumption of a modifying and significant form of behavior. When the marsupial invades the arboreal ecologic niche it converges to the facies of the arboreal rodent. When the reptile and the mammal assume the habit of flight they converge toward the avian facies. But *Oxyphysis* and its living relatives and presumably likewise its ancestors live in precisely the same ecologic niche with *Oxytoxum* to which it has converged in all its adaptive structural features in such minute detail and to such a striking degree of similarity. There is in this instance of convergence no evidence either of a modifying mode of behavior or the invasion of a new ecologic niche to initiate and complete this convergence of a dinophysid toward a peridinioid type, and of the operation of natural selection in the accomplishment of this change.

It might be proposed that *Oxyphysis* is a hybrid between a species of *Oxytoxum* and some dinophysid, with the result that it has the skeleton of the Dinophysoidae and the facies of *Oxytoxum*. The difficulties with this proposal are twofold. First, such a distribution of parental characters in the hybrid is startlingly unique. In the second place, sexual reproduction is not as yet established on cytological or other evidence in the Dinoflagellata.

The other suggestion is that the visible structural modifications which brought about this convergence and appear to have no differential survival value, are associated with some other factor, which, because of either linkage in the genetic sense, or because of the multiple aspects produced by single genes (see Holmes, 1909, 1915), has such a value but is not visible or at least not detected. Sturtevant (1925) has recently made this proposal to explain orthogenetic series in which differential survival values are not apparent and the differential characters which separate the species are not clearly of utility, or of differential utility.

Such a proposal does not seem to lend itself to either observation or experiment. Such associated physiological characters will always afford a final and safe retreat from these difficulties for the strict selectionist. Furthermore, in our particular instance, the factors involved include nearly the total complex of visible structures of the organism which constitute the *Oxytoxum*-like facies. The Dinoflagellata in all cases thus far examined have a considerable number of chromosomes. Linkage would seem to necessitate the assemblage of all factors constituting form control in a single one of the presumably numerous chromosomes. To explain this case of rather complete convergence by linkage with some unknown factor or diversity in the expression of genes seems to close the door to further analysis, or at least not to open one.

There remains a further concept of the underlying basis for this challenging phenomenon; namely, that we have to do here with a definite organ-forming substance, or complex of such definite chemical substances, whose molecular and supra-molecular aggregates constitute the structural basis for this phenomenon of convergence. With its evolutionary origin natural selection may have had nothing to do, and with its persistence it exercises, to use a teleological phrase, only a permissive function, having as much and no more causal relation to this phenomenon than it has with the occurrence of the amorphous, liquid, or crystalline forms of sulphur, or the individual units of a benzine series.

An inspection of the structural features of the skeletal systems of the Protista, such as the diatoms, Silicoflagellata, Coccolithophorida, Dinoflagellata, and Radiolaria, cannot but impress one with the seeming non-survival value of the interspecific differences which characterize the truly vast array of species in these groups. These species often occupy the same or similar habitats, frequently exist in great numbers of individuals, and are seemingly equally well adapted in many instances to live and to maintain themselves together. In any event, they are sufficiently well adapted to have survived.

Another feature which impresses one who inspects these skeletal structures, which give form, shape, symmetry, and interspecific differentiation to this array of evolutionary development at or near the roots of the tree of life, is the ever-recurring evidence of the play of forces which find expression in geometrical patterns, such as might be expected in supra-molecular aggregates in colloidal substances. For instance, trace the emergence of the reticular structure on the initially homogeneous skeleton of a newly forming valve of *Oxyphysis*. In figure 3 this valve is represented, at least in part, by the expanding, intercalary zones which will form respectively the right and left halves of the skeletons on the inner faces of the two daughters as they separate at binary fission. This homogenous substance is converted into the reticulate surface of figure 1 by the emergence of faint polygons, whose boundaries branch peripherally and spread over the face of the newly forming skeleton, which is moulded over separating cytosomes. These reticulations grow heavier with age; they may be resorbed as in the regulatory phenomena when the skeleton is reduced in area during rapidly repeated fissions, and they tend to be heavier in colder than in warmer waters. The environmental conditions and the internal states of the organism both modify them. The patterns are different in different species in the same genus. One cannot escape the conclusion that in the *skeletal structures* of the Dinoflagellata and other Protista we see the morphological expression of specific organ-forming substances whose chemical interrelationships afford the real basis for what we call the genetic resemblances of the speciating system. The interspecific differences seem therefore to be based fundamentally upon such different but related substances or even related groups of such interacting substances. If this basis be really that which causes the surface patterns of the skeleton of *Oxyphysis*, why may not also other supra-molecular interrelations of their aggregates be in like manner the basis for the system of plate patterns which characterize the genera and whose various mouldings into specific shapes differentiate the species of these genera?

The evolutionary concept of the development of the Dinoflagellata necessitates the divergence of the two tribes Dinophysoidae and Peridinioidae at some level from a common ancestry. They both show at binary fission the cleavage of the skeleton into two moieties as in *Prorocentrum* (Adiniferidea) . In the former the suture line is vertical, in the latter oblique. In the former there are but two horizontal sutures and three zones of plates. In the latter there are four horizontal suture lines and five zones of plates. There is thus evidence of a common basic organization evolving in two major lines. The ancestral organization had the structural basis to evolve in both directions in an epigenetic fashion. Perhaps the convergence of *Oxyphysis* to *Oxytoxum*-like facies is only the belated emergence of this primitive structural capacity, called forth by some interaction of the internal supramolecular state of the organism with the functioning or the environment of the organism. The clue to the meaning of this instance of convergence would then be sought neither in genetics nor in the origination of adaptations by the progressive action of selection, but rather in the mechanisms of organ-forming substances, if there be such substances.

Perhaps we shall make more progress in our understanding of the drama of evolution, at least in these, the most primitive essays in specific efflorescence, if we turn our inquiries and experiments toward the actors in the play rather than to the anthropomorphic stage director, who, we have reason to believe, sometimes rings down the curtain ere the act is completed.

SUMMARY

A new genus and species of the Dinophysoidae, *Oxyphysis oxytoxoides,* is described from the marine plankton at Loring, Alaska, and in the San Pedro Channel, off the California coast.

This genus has the plate structure of the Dinophysoidae and is related to the genera *Dinophysis* and *Phalacroma* in that tribe. It has the facies, size, shape, proportions, and degree of development of the organs of flotation of the genus *Oxytoxum* of the tribe Peridinioidae. It resembles certain species of *Oxytoxum* in a considerable number of details of structure.

This convergence does not seem to be the result of hybridization or of differential adaptive changes, induced by environment and preserved by selection because of survival value. The suggestion is made that it is rather the result of organ-forming substances whose molecular and supra-molecular organization or capacities for epigenetic organization as an interacting system have found expression rather suddenly at a high level in the speciating complex of the Dinophysoidae in the *Oxytoxum* facies, which has evolved elsewhere only in the diverging tribe Peridinioidae. The resemblance of *Oxyphysis* to the genus *Oxytoxum* may have had its origin in the potentialities of these organ-forming substances, which made possible the derivation of the two tribes from a common ancestry and which have not been eliminated in the speciating processes.

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DESCRIPTION OF PLATE

PLATE 18

All figures are from camera lucida drawings of specimens preserved in formalin.

Oxyphysis oxytoxoides nov. gen., nov. sp. All from Loring, Alaska, September 15, 1905.

Figures 1 to 4. × 1167.

Fig. 1. Lateral view of the right face showing surface structure, sulcus with low right and higher and longer left sulcal list with fission rib and enclosed flagellar pore. Terminal blades on both apex and antapex.

Fig. 2. Lateral view of left face of another individual with terminal blade only at the apex on the left valve (see fig. 3). Surface structure omitted.

Fig. 3. Same individual as in figure 2, in a megacytic phase. The thecal wall is splitting along the sagittal suture from the posterior end anteriorly parting the two valves. The megacytic phase is indicated by the great width of the intercalary zones on either side of the suture. These are finely striate by oblique, close-set parallel lines which run from the suture to the parasagittal lines near the margins of the figure. The intercalary zone is otherwise as yet undifferentiated. The surface markings beyond the parasagittal line are not drawn.

Fig. 4. Ventral view of a third individual with very narrow intercalary zones and terminal blades on both apex and antapex on the right (presumably the older) side. Note sagittal suture, parasagittal lines, right and left sulcal lists, the latter with the double fission rib very near the girdle, flagellar pore in the sulcus, and ventral pore in the left parasagittal line towards the apex. Surface structure omitted.

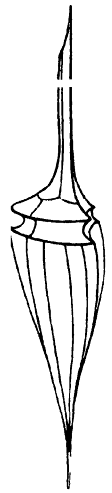
Figures 5 to 8, species of *Oxytoxum*

Fig. 5. *Ovytoxum subulatum* Kofoid (1907, pl. 10, fig. 62). × 470. Lateral view of right face. Compare with fig. 1 in apices, contours of epitheca and hypotheca, expansion of dorsal shoulder of the epitheca, and indented girdle.

Fig. 6. *Oxytoxum challengeroides* Kofoid (1907, pl. 10, fig. 65). × 780. Ventral view. Compare with figure 4, in proportions of epitheca, girdle, and hypotheca. Note the relatively blunter apices in this ventral view in *Oxyphysis*.

Fig. 7. *Oxytoxum compressum* Kofoid (1907, pl. 10, fig. 63). × 368. Oblique view of left face. Compare with figures 1 and 2. Note ventral deflection of antapex and extension of sulcus onto the epitheca.

Fig. 8. *Oxytoxum curvicaudatum* Kofoid (1907, pl. 10, fig. 61). × 368. View of left face. Note ventral deflection of antapex.



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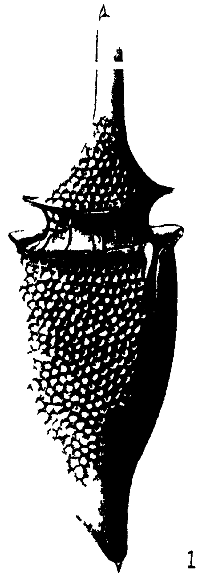

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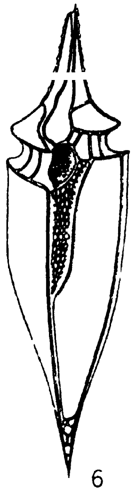

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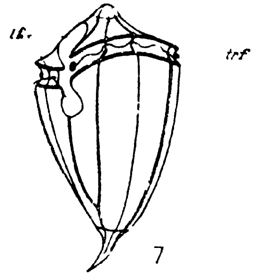

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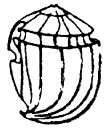

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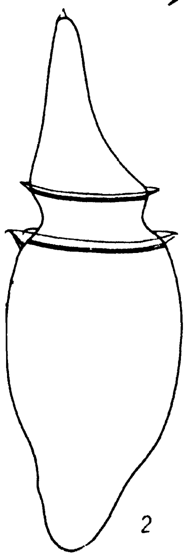

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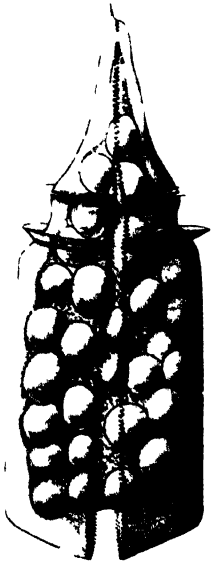

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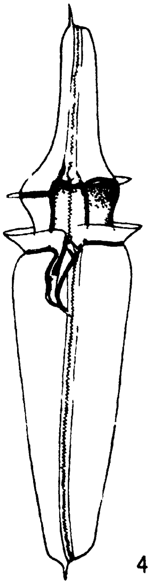

4

MITOCHONDRIA IN EUGLENA GRACILIS KLEBS

BY

DAVID CAUSEY

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY
Vol. 28, No. 11, pp. 217–224, plates 19–20
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MITOCHONDRIA IN EUGLENA GRACILIS KLEBS

LY

DAVID CAUSEY

CONTENTS

	PAGE
Introduction	217
Technique	217
Morphology of the motile and encysted phases	218
Mitochondria in the motile and encysted phases	219
Summary	221
Literature cited	243
Explanation of plates	222

INTRODUCTION

With the exception of *Cryptomonas* and *Chilomonas paramecium* (Fauré-Fremiet, 1910), the literature on the mitochondria in flagellates appears to deal exclusively with parasitic forms. It has therefore been considered advisable to examine a typical free-living flagellate and to determinne to what extent data on the mitochondria in parasitic flagellates are valid among free-living flagellates. The organism used, *Euglena gracilis* Klebs, has been carefully studied by Zumstein (1899) and Ternetz (1912) from viewpoints of morphology and physiology, and is accordingly of peculiar value for further study. I am indebted to Mr. A. S. Campbell, of the Department of Zoology, University of California, for cultures of this flagellate. The study has been made under the direction of Dr. C. A. Kofoid, to whom I am indebted for suggestions and criticisms.

TECHNIQUE

The technique used was the osmic acid fixation and modified haematoxylin staining as previously reported (Causey, 1925), with the following modifications: In place of smear preparations, the centrifuge was used throughout the technique until the organisms were placed in balsam. It was found necessary to bleach the chromatophores with potassium permanganate and oxalic acid in order to obtain clear and sharply stained organisms. Sunlight, alcohol, and hydrogen peroxide were tried unsuccessfully for this purpose. Without bleaching, the preparations obtained have the chromatophores preserved with apparently no change in color or shape, but the other details are obscured. As reported for *Leishmania* (Causey, 1925*b*) the neuromotor system is not well stained in preparations destained for mitochondria. The fibrils of the neuromotor system lose the stain more rapidly than do the other cytoplasmic structures.

MORPHOLOGY OF THE MOTILE AND ENCYSTED PHASES

Euglena gracilis Klebs is a typical representative of the genus, and for details of its morphology the reader is referred to the papers of Zumstein (1899) and Ternetz (1912). My observations are in agreement with theirs. According to these investigators four forms may be recognized in this species: a normal green form (*A*), a hyaline dark form (*B*), an intermediate form (*C*), and a hyaline light form (*D*). The green form (*A*) is the form encountered in nature; it is holophytic, and may grow both in media containing only inorganic matter and media containing only organic matter. The other forms seem to be cultural varieties and to vary with the cultural factors. The specimens used for this study were of the type *A*, the normal green organism. An occasional specimen of form *D* has been observed in the cultures, but is relatively rare. According to Ternetz, the chromatophores vary in number from 1 to 30. Those used for this study show from 10 to 15, the average being about 12 per organism. In the organisms figured (pls. 19 and 20) the chromatophores are not so wide as those figured by Zumstein and by Ternetz, and also not as wide as those observed in the living organism. This difference is interpreted as due to the bleaching method employed, and is not apparent in the unbleached specimens. The bleaching method used does not invalidate the study of the mitochondria, as it is commonly used in mitochondrial technique to correct excessive mordanting with osmic acid and bichromate (Cowdry, 1918). Unbleached specimens show the mitochondria and other structures, but less clearly. According to Ternetz, division occurs about every 18 hours in a good culture. In my own material dividing forms are very rare, due perhaps to rapidity of the whole process. The cysts found show the essential features of the motile phase with the exception of the neuromotor system, are more or less rounded, and, in my material, do not show any evidences of division occurring in them. Single organisms have been observed to escape from the cysts.

Pyrenoids are not visible in the chromatophores, but are numerous in the cytoplasm, and in most cases are surrounded by a vacuole filled with paramylum, and "appear to be the centres of the production of amyloid substance" (Minchin, 1922). The significance of those which are not surrounded by paramylum is discussed later on.

MITOCHONDRIA IN THE MOTILE AND ENCYSTED PHASES

The mitochondria present in the motile and encysted phases are spherical in form (pl. 19, figs. 1–4, and pl. 20, figs. 5–9). The number present varies but is always small. In the motile phase from 10 to 25 have been observed, with an average of about 16 per organism. The number in the encysted phase is less, being approximately half that found in the motile phase. When the relative amount of cytoplasm in the two phases is considered, it seems probable that there is a more or less definite ratio between the number of mitochondria and the volume of cytoplasm, as was shown for *Leishmania* (Causey, 1925*b*). No association or close proximity with other cytoplasmic structures is apparent. This lack of association with other cytoplasmic structures has been demonstrated in parasitic flagellates—*Trypanosoma* (Shipley, 1916), *Leishmania* (Causey, 1925*b*).

In previous papers (Causey, 1925*a*, *b*) it has been shown that the mitochondria are associated with the metabolic activities of the organism, and that the rod-shaped mitochondria take part in anabolic processes, while the spherical mitochondria take part in the catabolic activities. In *Leishmania*, where the food is absorbed from the surrounding medium, and anabolic activities are reduced to the minimum, rod-shaped mitochondria were present only during times of unusual anabolic activity. In *Euglena gracilis*, which has chromatophores, the food material is elaborated from inorganic materials by photosynthesis differing in no known way from that of green plants. The anabolic processes when carried out in this way do not appear to give rise to rod-shaped mitochondria. Regardless of the way the food materials are obtained, and regardless of the type of anabolic activity, the catabolic activities in organisms are of the same fundamental nature, and under such conditions the spherical mitochondria may be demonstrated.

It has already been mentioned that pyrenoids are found in the cytoplasm, which are not surrounded by paramylum (pl. 18, fig. 4; pl. 19, figs. 5, 6, 9). In so far as shape and staining indicate, there is no difference between them and the mitochondria except that of size, the pyrenoids being larger. There is sufficient difference in the size of the mitochondria to make possible the arrangement of a morphological series showing the transition of the one into the other. Alexeieff (1917) has pointed out that the parabasal body in parasitic flagellates is of mitochondrial origin, and that the mitochondria secrete glycogen. Boeck (1919) has shown that in *Giardia microti* the parabasal body secretes glycogen. The secretory function demonstrated for mitochondria in parasitic flagellates, and the morphological resemblance of the pyrenoids to mitochondria, make it probable that the pyrenoids are derivatives of the mitochondria in such free-living flagellates as *Euglena gracilis.* Such a conception, first advanced by Lewitsky (1910) and by Pensa (1910) for the origin of chloroplasts and leucoplasts, has been extended by numerous workers to cover the origin of plastids in general in plants. This conception need not carry with it the idea of actual chemical change in the mitochondria which Cowdry (1918) considers improbable, but rather Regaud's conception that the mitochondria "play the part of plasts choosing and selecting substances from the surrounding cytoplasm, condensing them in their interior into infinitely diverse products" (*ibid.,* p. 103).

In comparing the data regarding the mitochondria in flagellates, whether parasitic or free-living, no essential differences are apparent. There appears to be a more or less well defined ratio between the volume of the cytoplasm and the number of mitochondria. In each type of flagellate spherical mitochondria are present and are to be correlated with the katabolic activities of the organism. In each, the secretory bodies in the cytoplasm, the parabasal in parasitic forms, and the pyrenoids in the free-living forms, are probably of mitochondrial origin, and secrete substances of close chemical relationship, glycogen and paramylum respectively. There appears to be no evidence in either parasitic or free-living forms for the persistent identity of the mitochondria, which appear to arise *de novo* under metabolic influences and to disappear after the completion of their function.

SUMMARY

1. The mitochondria present in *Euglena gracilis* are spherical in form and are correlated with the katabolic activities of the organism.

2. It is probable that a definite ratio exists between the volume of cytoplasm and the number of mitochondria.

3. The pyrenoids, which secrete paramylum, indicate by their staining reactions that they are of mitochondrial origin, and comparable to the parabasal bodies in parasitic flagellates.

4. The mitochondria arise *de novo,* and do not have a persistent identity during the life of the organism.

5. The data regarding the mitochondria in free-living flagellates are in agreement with the data for mitochondria in parasitic flagellates.

For literature cited see page 243.

EXPLANATION OF PLATES

All figures of *Euglena gracilis* Klebs, fixed in osmic acid and stained in modified haematoxylin. All of the specimens figured were prepared at the same time, so the figures show the difference in stainability. × 3000.

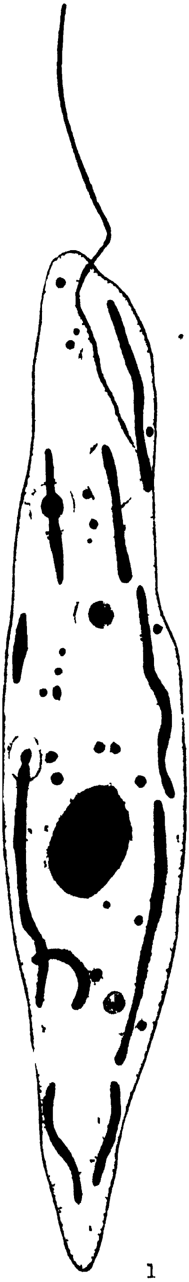
PLATE 19

Fig. 1. Specimen with granular cytoplasm, mitochondria numerous and evenly distributed.

Fig. 2. Specimen with cytoplasm highly vacuolated, mitochondria few. Chromatophores lightly stained.

Fig. 3. Specimen with cytoplasm intermediate between proceeding figures. Few pyrenoids. Mitochondria showing transitional stages toward development of pyrenoids.

Fig. 4. Specimen with numerous pyrenoids and mitochondria. One pyrenoid without surrounding paramylum at anterior end.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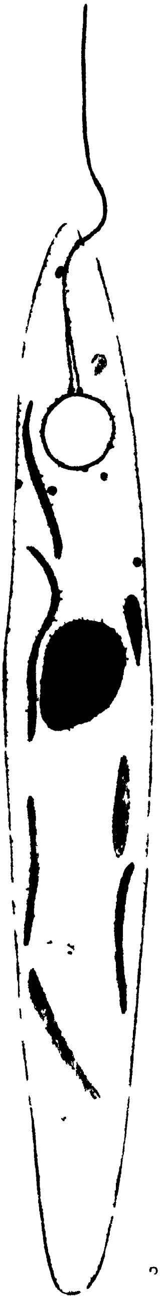
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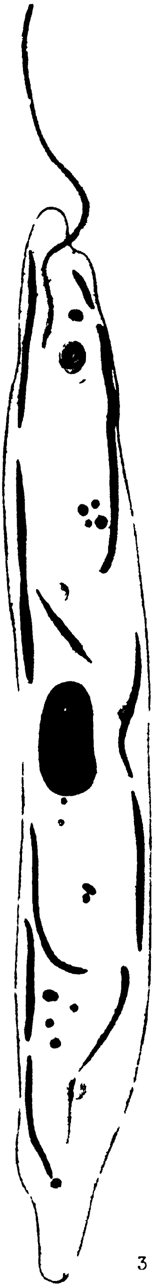
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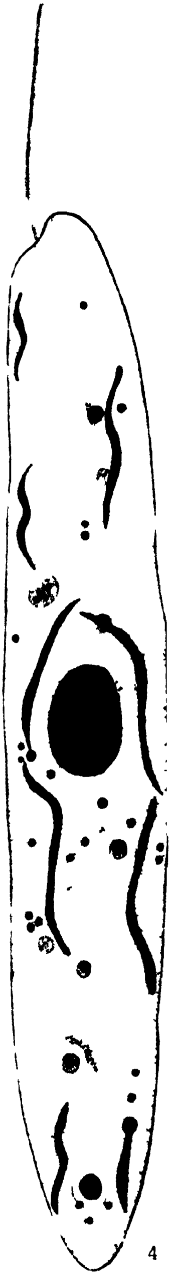
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PLATE 20

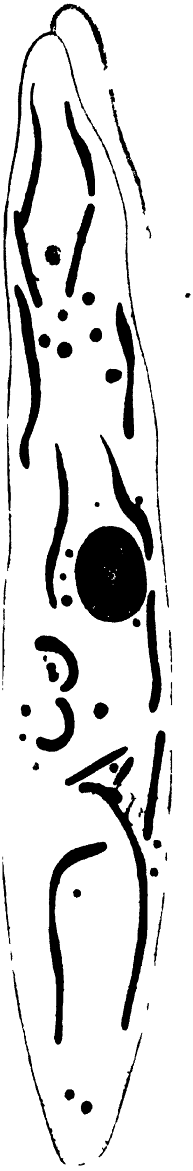
Fig. 5. Specimen with numerous mitochondria and pyrenoids not surrounded by paramylum. Numerous transitional stages.

Fig. 6. Cyst showing mitochondria, pyrenoid with and without paramylum. Cytoplasm faintly stained.

Fig. 7. Cyst with darker staining cytoplasm, with pyrenoids surrounded by paramylum, and mitochondria.

Fig. 8. Cyst nearly ready to open. Cytoplasm darkly stained. With numerous mitochondria.

Fig. 9. Small specimen with granular cytoplasm and numerous mitochondria. Pyrenoids with and without surrounding paramylum.



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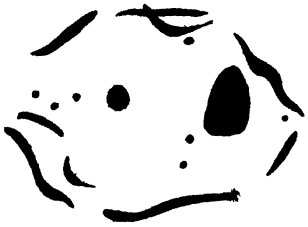

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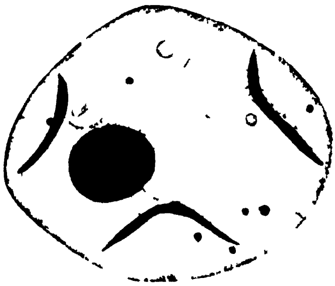

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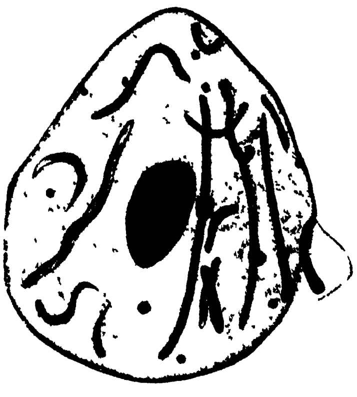

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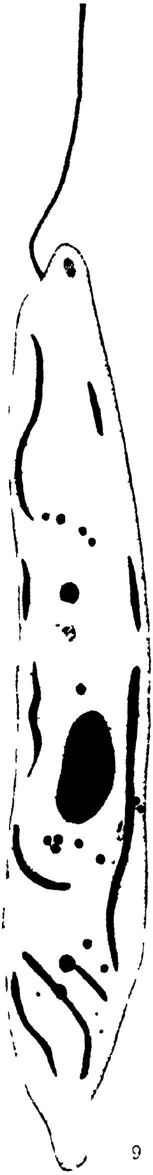

9

MITOCHONDRIA IN NOCTILUCA SCINTILLANS (MACARTNEY 1810)

BY

DAVID CAUSEY

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MITOCHONDRIA IN NOCTILUCA SCINTILLANS (MACARTNEY 1810)

BY

DAVID CAUSEY

CONTENTS

	PAGE
Introduction	225
Technique	226
Mitochondria and their relation to the metabolic activities	227
Summary	229
Literature cited	243
Explanation of plate	230

INTRODUCTION

The sole reference to the presence of mitochondria in *Noctiluca* appears to be that of Fauré-Fremiet (1910). He figures a tentacle of the organism which shows numerous spherical mitochondria, and mentions in his explanation of the figure the occurrence of numerous mitochondria in the ectoplasm. The occurrence of spherical mitochondria in so active a structure as the tentacle of *Noctiluca* is very significant, and a study of the mitochondrial content of the organism as a whole is therefore desirable. The present study is based on collections from San Francisco Bay, and confirms and extends Fauré-Fremiet's observations. It has been done under the direction of Dr. C. A. Kofoid, to whom I am indebted for both suggestions and criticisms. The morphology and relationships of *Noctiluca scintillans*, the type and only species of the genus, has been reviewed recently by Kofoid and Swezy (1921), whose monograph should be consulted for such information.

TECHNIQUE

Collections were made either at or just before high tide from both the Key System pier off Oakland, and by means of a motor boat from the waters off Cavallo Point at the inner part of the Golden Gate. The specimens obtained from the latter station appear more hardy, and do not collapse so much as do those obtained near Oakland. This difference is probably due to the fact that off Cavallo Point the water is fresh from the ocean and has not undergone any changes, while at the Key pier, farther within the bay, sufficient contamination by sewage has occurred to cause appreciable changes in the organism. In addition, the salinity off the Key pier is often from five to ten parts per thousand below normal.

The organisms obtained with a plankton net were drained into a pint jar and fixed immediately by adding osmic acid solution. The material so fixed was then taken to the laboratory, and after a few hours of the osmic acid fixation, was washed with distilled water and mordanted with potassium bichromate. After again washing with several changes of distilled water to remove the excess of potassium bichromate, the organisms were stained with iron-haematoxylin and mounted in balsam. No attempt was made to separate the desired organisms from the other material in the catch. Attempts to obtain perfect, uncollapsed specimens in balsam were unsuccessful. The organisms are so delicate that each change of fluid causes additional ones to collapse, and by the time the material is in balsam few are in condition for study. Neither centrifuging very carefully nor allowing the organisms to settle before changing the fluid increased the number of successful preparations. The study has been based on the relatively few specimens in approximately normal condition present in balsam preparations. After prolonged mordanting with potassium bichromate, the mitochondria are readily seen in the unstained organism, and such material has been made the basis of comparison with the balsam preparations. No differences are observable. The figure of the whole organism (pl. 21, fig. 4) was made from a mordanted but unstained specimen.

MITOCHONDRIA AND THEIR RELATION TO THE METABOLIC ACTIVITIES

The cytoplasm in *Noctiluca* is remarkably clear, and even in the stained preparations destained to show the mitochondria, the interstices of the strands of thickened cytoplasm show practically no visible structure. The food vacuoles present are almost without exception found in the more central portion of the organism. Here the cytoplasm is more concentrated and stains darker. The food appeared to be almost exclusively diatoms, and no apparent preference for any particular diatoms was shown. All the more common species of marine diatoms have been observed in the food vacuoles. *Noctiluca* seems to have remarkable ability in ingesting diatoms of approximately its own size, and occasionally a specimen is encountered containing a diatom so large that only a thin periphery of cytoplasm and the tentacle are visible. In all cases observed, a vacuole is formed about a single diatom, although several such vacuoles may be present in a single organism (pl. 21, fig. 4). The cytoplasm not in the vicinity of the vacuoles contains long anastomosing strands of thickened cytoplasm, the result of the streaming movements evident in the living organism.

The mitochondria present in *Noctiluca* are of two types, rod-shaped and spherical. The rod-shaped mitochondria are found, without exception, in the concentrated cytoplasm in the vicinity of the food vacuoles (pl. 21, figs. 1, 3, 4). This relationship of food vacuoles and rod-shaped mitochondria is identical with that found in *Endamoeba gingivalis* (Causey, 1925). They are not so numerous as was the case in *E. gingivalis*, probably because of the difference in quantity of food material actually present in the vacuole. There is relatively little food material present in a diatom, as a considerable portion of its bulk is made up of the non-nutritive test and vacuoles in its cytoplasm.

The spherical mitochondria are the more numerous, and are distributed throughout the remaining portion of the cytoplasm (pl. 19, figs. 4, 6). In the majority of cases the spherical mitochondria are distributed along the anastomosing strands of cytoplasm, particularly along those of more recent formation (pl. 21, figs. 4, 6). The latter strands are less dense and stain lightly. Those not associated with the cytoplasmic strands are usually in a more or less linear order, suggesting that either a strand was in the process of formation at the time the organism was killed, or that the cytoplasm had been destained sufficiently to render the actual strand invisible. The spherical mitochondria are most numerous in the tentacle and appear to be associated with the cross-striations of the latter (pl. 21, fig. 2). I find them not so numerous and considerably larger than they appear to be in Fauré-Fremiet's (1910) material. The terminal concentration of mitochondria shown in his figure is not evident in mine.

As has been reported in previous papers (Causey, 1925), in the Protozoa the mitochondria appear to be directly correlated with the metabolic activities of the organism. In regions of anabolic activities the rod-shaped mitochondria are to be found, while in the regions of katabolic activity the spherical mitochondria are present. In *Noctiluca,* as in *Endamoeba gingivalis,* the rod-shaped mitochondria are found to be associated with the food vacuoles where the anabolic activities are high. In the remaining portions of the cytoplasm, where katabolic activities are pronounced, the spherical mitochondria are to be found. The formation of pseudopodia was shown in *Endamoeba gingivalis* to be associated with the spherical mitochondria, and their formation to be due to solation of the cytoplasm in the vicinity of the mitochondria. A comparable condition is present in *Noctiluca,* where the spherical mitochondria are associated with the strands arising from the streaming of the cytoplasm. Equally conclusive is the presence of a relatively greater number of spherical mitochondria in the tentacle. This is probably of no importance as an organ of locomotion, but serves as an active means of getting food. The katabolic activities are consequently high, and, as is to be expected, are indicated by the relatively greater number of spherical mitochondria.

A condition of the cytoplasm which appears to be abnormal is occasionally found (pl. 21, fig. 5). The cytoplasm is nearly filled with spherical bodies of various sizes. The larger ones are clearly food bodies, and the intermediate ones appear to be of the same nature. Among these are found numerous mitochondria and somewhat larger bodies, which from their staining reactions appear to be fat bodies. In such cases there is no central concentration of the cytoplasm containing the food vacuoles. This condition is probably to be interpreted as a moribund condition, possibly due to the ingestion of more food material than can be utilized by the organism.

SUMMARY

1. The mitochondria present in *Noctiluca scintillans* are of two types: rod-shaped and spherical.

2. The rod-shaped mitochondria are always associated with the food vacuoles and are correlated with the anabolic activities of the organism.

3. The spherical mitochondria are present in the remaining portions of the cytoplasm, including the tentacle, and are correlated with the katabolic activities of the organism. In the bulk of the cytoplasm they are associated with the strands arising from the streaming of the cytoplasm, and in the tentacle they are associated with the cross-striations.

4. The data are in accord with comparable phenomena in *Endamoeba gingivalis.*

For literature cited see page 243.

EXPLANATION OF PLATE 21

Figures of *Noctiluca scintillans* (Macartney 1810). All figures × 1035 except figure 4, which is × 425.

Fig. 1. Food vacuole containing diatom in last stage of absorption. Rod-shaped mitochondria in vicinity.

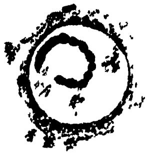
Fig. 2. Tentacle showing spherical mitochondria and cross-striations.

Fig. 3. Food vacuole with numerous rod-shaped mitochondria in vicinity.

Fig. 4. Typical organism showing food vacuoles in the central portion of cytoplasm. Rod-shaped mitochondria in vicinity of vacuoles. Peripheral portions of cytoplasm with thickened strands and clear interstices, showing spherical mitochondria. Tentacle shows numerous spherical mitochondria. From mordanted but not stained specimen.

Fig. 5. Portion of moribund cytoplasm containing numerous food vacuoles, fat bodies, and mitochondria.

Fig. 6. Portion of normal cytoplasm, showing the relation of the spherical mitochondria to the thickened strands of cytoplasm.



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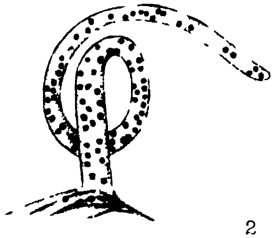

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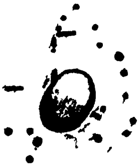

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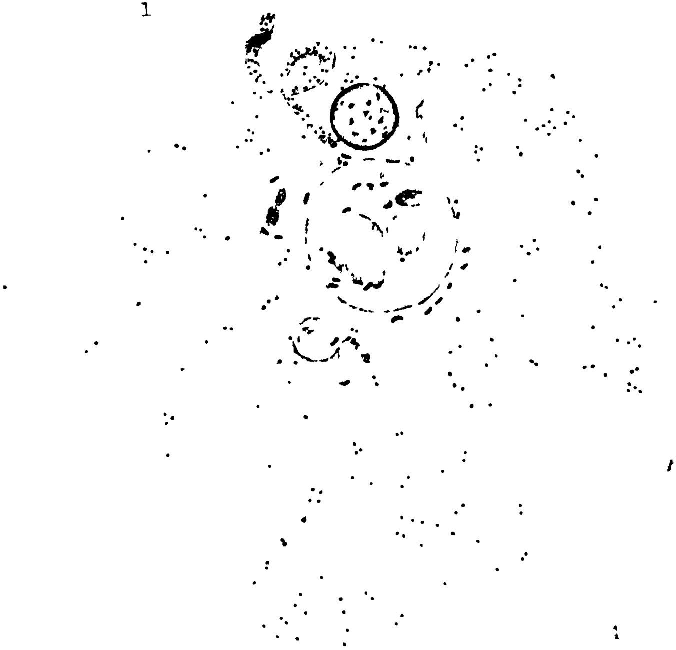

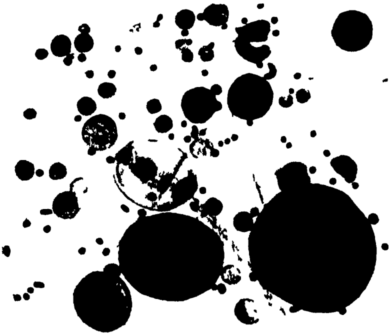

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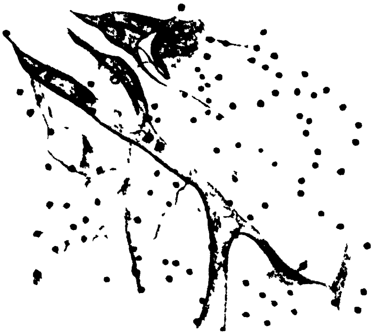

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MITOCHONDRIA IN CILIATES WITH ESPECIAL REFERENCE TO PARAMECIUM CAUDATUM EHR.

BY

DAVID CAUSEY

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CONTENTS

	PAGE
Introduction	231
Technique	232
Mitochondria in ciliates	232
Mitochondria in *Paramecium caudatum*	233
In the vegetative organism	234
During binary fission	235
During conjugation	236
Significance of the granules of the neuromotor system	238
Summary	342
Literature cited	243
Explanation of plates	246

INTRODUCTION

Perhaps no protozoan forms have been the subjects of so much investigation in connection with problems of genetics, physiology, and general life-history as have the two common species of *Paramecium*, *P. caudatum* and *P. aurelia*. Furthermore, these species are in practically universal use in biological laboratories as a means of introducing students to the study of the Protozoa. It has therefore been considered advisable to investigate the presence and behavior of the mitochondria in the more common of these two species. The present study is based mainly upon *Paramecium caudatum*, with some references to other ciliates commonly encountered in fresh-water aquaria. The work has been under the direction of Dr. C. A. Kofoid, to whom I express my appreciation of his kindly interest and criticisms, and of the many suggestions which made the work possible. The clone used for the investigation is from the cultures of Dr. G. H. Ball, who has recently reported on the behavior of this conjugating race (Ball, 1925). The other ciliates were obtained from cultures in the Department of Zoology of the University of California.

TECHNIQUE

Janus green *B* in a dilution of 1/10,000 has been used in the study of the living organisms. For fresh-water Protozoa it must be made up with either distilled water or with the culture medium itself. For permanent, stained preparations the organisms were handled throughout the technique in certrifuge tubes, except when destaining. The organisms were fixed in osmic acid solution, washed in distilled water, and slightly bleached with a few drops of hydrogen peroxide added to the distilled water. They were then mordanted in 5 per cent iron alum, and stained in Regaud's modified haematoxylin, dehydrated, and mounted in balsam. Counterstaining with eosin in 95 per cent alcohol gives a pleasing contrast. Care must be taken to avoid overstaining, as it is practically impossible to remove excess stain without having the organisms turn a muddy brown color which renders them worthless. For sections, the organisms were killed with osmic acid, dehydrated, imbedded in paraffin, and, after sectioning, stained as for the whole organism.

MITOCHONDRIA IN CILIATES

The presence of mitochondria in ciliates has been reported by several investigators, of whom Fauré-Fremiet is the most prominent. A list of the species is given in Cowdry's monograph on mitochondria (1918). To this list this paper adds the following: *Chaenea teres* Duj. (pl. 24, fig. 13), *Microthorax sulcatus* Engelmann (pl. 24, fig. 14), *Colpoda saprophylla* Stokes (pl. 24, fig. 15), *Stylonychia pustulata* Ehr. (pl. 24, fig. 16), and *Euplotes patella* Ehr. (pl. 24, fig. 17).

Fauré-Fremiet (1910) studied the mitochondria in *Paramecium caudatum,* and gives several figures of them. The only other reference to the mitochondria in *Paramecium* appears to be that of Young (1924), who mentions their presence in his material but does not figure them.

In his discussion Fauré-Fremiet (1910) mentions that in all the ciliates the mitochondria show a remarkable uniformity of character and distribution. This appears to be true for all the ciliates thus far investigated. In ciliates which do not have definite food vacuoles present at the time of fixation, the mitochondria are uniformly distributed throughout the cytoplasm (pl. 24, figs. 13, 15) with some tendency toward an accumulation in the peripheral portions (pl. 24, figs. 14, 17). Most characteristic of the ciliates is the large number of rod-shaped mitochondria and the relatively small number of spherical mitochondria present. Occasionally a specimen is found in which only spherical mitochondria are present (pl. 24, fig. 16). This is of relatively rare occurrence, for the majority of the specimens show the rod-shaped mitochondria. As was suggested for similar conditions in *Noctiluca scintillans* (Causey, 1925*d*), this appears to be a moribund condition, and to be attributable to a disturbed metabolic activity. In such forms as *Microthorax sulcatus* and *Euplotes patella,* where the more central portion of the cytoplasm is vacuolated and stains intensely, it is difficult to determine the mitochondria in this region (pl. 24, figs. 14, 17). In view of the conditions found to be present in *Paramecium caudatum,* it seems probable that this difference in the distribution of the mitochondria is only apparent, not actual, and is due to varying stainability of the cytoplasm.

MITOCHONDRIA IN *PARAMECIUM CAUDATUM*

Fauré-Fremiet (1910) appears to have found only spherical mitochondria in his material. Part of this was fixed in osmic acid and stained in eosin. The rest was similarly fixed, but stained in haematoxylin or gentian violet. Although confusion with other cell inclusions might easily follow the use of eosin as a stain, I am not able to state any reasons for his results with the haematoxylin. This was presumably ordinary haematoxylin, which, according to Gatenby (1921, p. 324) is "quite as good" as the modified haematoxylin used in my own studies. From his figure of a cross-section of the organism through the macro- and micronuclei (fig. xvii, p. 512) one is led to conclude that he interpreted all rod-shaped bodies as trichocysts. This is a rather improbable mistake, however, as the shape and size of the trichocysts are quite different from those of the rod-shaped mitochondria. According to my own observations, the trichocysts are found only in the fibrillar ectoplasm. I do not find the trichocysts placed in whorls as reported by Rees (1922); instead, they are irregularly distributed.

In the vegetative organisms.—

The number and distribution of the mitochondria in *Paramecium caudatum* vary with the metabolic activities of the organism. Usually they are found exclusively in the region of cyclosis, around and between the food vacuoles (pl. 22, figs. 1–6). In an organism which has been feeding actively to an unusual extent, the mitochondria are found not only in this region but in the peripheral cytoplasm as well. The cross-sections of the organism figured (pl. 23, figs. 7–12) show such a condition. The specimen shown on plate 20, figure 1 shows the typical relations and distribution which are found in the majority of specimens examined.

When *Paramacium* is examined in a medium containing Janus green *B*, the mitochondria are seen to stain very promptly. The majority of those visible in an organism at any time are rod-shaped and in the region where cyclosis occurs. The first indication of staining is in the region at the end of the cytopharynx, where, presumably, the stain is first absorbed. The bacteria which form the principal food of *Paramecium* in cultures stain very faintly with the Janus green, and contrast clearly with the sharply stained mitochondria. As cyclosis continues and more stain penetrates the organism, the various food vacuoles are very sharply differentiated from the other cytoplasmic organelles by the densely stained mitochondria which surround them. Other mitochondria are to be seen scattered in the cytoplasm between the food vacuoles. Although careful focusing makes clear the position of the mitochondria about and not in the vacuoles in either living or fixed material, reference to sections (pl. 23, figs. 7–12) makes it perfectly clear that the stainable bodies, the mitochondria, are closely clustered about the food vacuoles.

Khainsky 1910) has investigated the fate of the food vacuoles in *Paramecium* and recognizes the following stages. In the early stages of a food vacuole, the bacteria contained in it are dispersed and stain lightly. Next follows a stage in which the bacteria become clumped together to form 'food-balls' which stain intensely. These are at first numerous, but become fewer in number as absorption occurs. In the final stages the food balls disappear, and an indistinctly stainable residue is left. From this residue the crystalloids commonly present in the cytoplasm are apparently derived. So far as my observations extend, they are in agreement with his conclusions. The behavior of the mitochondria in relation to the food vacuoles as seen in vitally stained organisms indicates that the mitochondria arise *de novo* in the vicinity of the terminal end of the cytopharynx, about the newly formed food vacuole, and accompany it throughout its course in cyclosis. The presence of the lesser number of mitochondria between the vacuoles, but in the region of cyclosis, indicates that processes similar to those in the vacuoles (anabolic) are continuing in these portions of the cytoplasm. The general distribution of the vacuoles and the mitochondria in the non-dividing organism is shown in plate 22, figure 1. There can be no question regarding the lack of independent motility on the part of the mitochondria, as in the vitally stained organism they can be followed throughout cyclosis, passively accompanying the food vacuoles, and never showing any independent motility.

In previous papers (Causey, 1925 *a–d*), evidence has been presented for the correlation of the rod-shaped mitochondria with the anabolic activities of the organism. In *Endamoeba gingivalis* and in *Noctiluca scintillans* it was shown that the rod-shaped mitochondria are definitely associated with the food vacuoles, and are to be found in no other regions. The close association of the rod-shaped mitochondria in *P. caudatum* with the food vacuoles offers further evidence for the truth of this interpretation. The conditions present in *Paramecium* during binary fission and conjugation, when the cytostome and cytopharynx dedifferentiate and disappear, when feeding ceases, and anabolic activities are reduced, should offer additional evidence pertinent to this conception.

During binary fission.—

The changes in *Paramecium* during binary fission have long been known and need not be summarized. The changes of importance to the following discussion are that the cytostome and cytopharynx dedifferentiate and disappear, and that during this time no feeding is possible until the daughter organisms develop these structures anew. The metabolic activities in the vacuoles containing food at the beginning of the fission continue until the contents are absorbed.

Fauré-Fremiet (1910) has reported that in *Carchesium polypinum* the mitochondria divide simultaneously with the micronucleus. If demonstration supports this report it will furnish considerable evidence for the continuity of the mitochondria as persistent cell organelles, and will be equally adverse to the *de novo* conception of the origin of the mitochondria. Because of the interest attached to such a question two figures of binary fission in *Paramecium caudatum* are given here (pl. 22, figs. 3, 6), showing very early and late binary fission. Stages have been available from the earliest phase in binary fission where the micronucleus migrates from the pocket in the macronucleus into the adjacent cytoplasm, on through its successive enlargement, elongation, and division, and the ultimate division of the macronucleus and cytoplasm. The mitochondria during these phases show no essential differences from the two stages figured. The association of the mitochondria with the food vacuoles and the cytoplasm in their immediate vicinity persists, and at no time does any evidence or indication of division appear. So far as the mitochondria are concerned, the difference between these stages and the non-dividing organism is merely that of food content. Feeding having ceased, the number of vacuoles is reduced, and consequently the actual number of mitochondria is somewhat reduced. Differences in the number of food vacuoles present at the time of binary fission lead to similar differences in the resulting stages, but these have no bearing on the process. In the early phase, where the micronucleus is elongating and a half passing to each end of the organism (pl. 22, fig. 6), the vacuoles are few in number and are surrounded by clusters of mitochondria. In late binary fission the same conditions are found (pl. 22, fig. 3). The conditions are entirely different from those shown for *C. polypinum* (Fauré-Fremiet, 1910, p. 525, fig. xxxii).

Cowdry (1918) has pointed out the difficulty of determining from prepared material whether observed appearances show "an actual multiplication by division or simply an approximation of originally separate mitochondria." Fauré-Fremiet's observations, however, were made on living material and are due possibly to the misinterpretation of other cytoplasmic inclusions as mitochondria. He used various vital stains—neutral red, methylene blue, and Bismarck brown—none of which is as specific as Janus green *B*. Janus green *B* did not come into use as a mitochondrial stain until the year after Fauré-Fremiet's paper was published (Bensley, 1911). Other investigators have reported the division of mitochondria, but no convincing evidence has been offered. In studies on the Protozoa of the different groups—amoeba, flagellates, and ciliates—I have thus far seen no indication of division of the mitochondria and am convinced that they arise *de novo* in response to metabolic conditions.

During conjugation.—

During conjugation, as in binary fission, there is no possibility of feeding by the organisms concerned until the act is completed. The same conditions present during binary fission hold, therefore, for the conjugating pair. Whatever food material is present in the vacuoles at the time of conjugation is eventually absorbed, independently of the reproductive processes under way. The mitochondria present in the conjugating pair (pl. 22, fig. 4) show the same spatial relation to the food vacuoles as is found in the non-dividing organism (pl. 22, fig. 1). The actual number of mitochondria present depends upon the number of food vacuoles present and their contents, and this, in turn, is dependent upon the feeding activities of the organism before conjugation. Thus, in an exconjugant immediately after separation from its coconjugant (pl. 22, fig. 2) the number of mitochondria present may be greater than in a conjugating pair (pl. 22, fig. 4). Other examples might be chosen to show the reverse. Regardless of the number of mitochondria present, however, it is evident that there is no relation to the conjugation phenomena occurring. Likewise, in one of the daughters resulting from conjugation and successive division, the mitochondria show identical spatial relations with the reduced number of food vacuoles (pl. 22, fig. 5). Here there has been no opportunity as yet to renew feeding, and the actual amount of food present in the organism is small.

It seems clear, in the light of the preceding evidence, that the rod-shaped mitochondria in *P. caudatum* are concerned with the metabolic activities of the organism, and more specifically with the anabolic processes taking place in the food vacuoles. This is in complete agreement with previous studies on *Endamoeba gingivalis* and *Noctiluca scintillans* (Causey, 1925*a* and *d*), and makes it probable that such a relation may be demonstrated in the Protozoa wherever vacuolar digestion occurs. This is in conformity with the theory suggested by Regaud (1909) and concisely summarized by Cowdry (1924) as follows:

> the mitochondria are said to play the part of plasts choosing and selecting substances from the surrounding cytoplasm, condensing them and transforming them in their interior into infinitely diverse products. . . . . But it is not necessary to endow the mitochondria with any wonderful anthropomorphic attributes, for they may equally well act in a more or less passive way, taking up materials by virtue of their peculiar constitution, or on account of physical forces acting on their surface. We can conceive of chemical changes arising: (1) by the addition of substances from the cytoplasm which enter into close combination and become integral constituents of the mitochondria; (2) by the mitochondria giving up to the cytoplasm certain of their normal constituents; and (3) by chemical dissociation and by chemical resynthesis within the mitochondria.

SIGNIFICANCE OF THE GRANULES OF THE NEUROMOTOR SYSTEM

In considering the granules which are components of the neuromotor system of ciliates and flagellates, certain morphological relations of the neuromotor system itself must be considered. In flagellates, where the more primitive type of neuromotor system is found, the following structures may be demonstrated: an extra-nuclear centrosome, which is usually on the nuclear membrane, and in morphological connection with the karyosome by means of a nuclear rhizoplast; in the cytoplasm, and at the anterior end of the organism in all cases of clear polarity, one or more blepharoplasts, usually designated as the primary, secondary, etc.; a rhizoplast connecting the centrosome and the primary blepharoplast, and when more than one blepharoplast is present the additional ones are serially connected with the primary blepharoplast by means of rhizoplasts; flagella, arising from the blepharoplasts, either single or multiple in number; and finally, connected with the neuromotor system may be demonstrated, in the parasitic flagellates, a parabasal body (= kinetonucleus), an axostyle, and an undulating membrane of the cytostome. Any or all of these additional structures may be absent.

The neuromotor system in ciliates is of the same fundamental type. There is, however, a neuromotor center from which fibers extend in definite patterns to all or restricted parts of the ectoplasm. These fibers extend to the rows of basal granules from which the cilia or their derivatives, the cirri, arise. Such structures as the parabasal body and the axostyle are not present in ciliates. Fundamentally, then, the neuromotor system of Protozoa consists of a varying number of granules, which are, however, constant for a species, arranged in an unvarying and definite order, connected with each other and a directive center—motorium or nucleus—by cytoplasmic fibers, while from each basal granule or blepharoplast a motor organelle—cilium or flagellum—arises.

To demonstrate the mitochondrial origin of the granules of the neuromotor system in Protozoa will require a technique, which, although greatly desired, is not as yet available. The mitochondria can be demonstrated with certainty using present methods, but means are not available for tracing the rather uncertain chemical changes from the phospholipin composition of the mitochondria into that of bodies of still unknown chemical composition. It is to be hoped that future work along the lines of the chemistry of the cell and its inclusions will make such investigations possible. At present one can only assemble the facts which point to such an interpretation. The first step in advance will probably be the development of a technique which will differentiate between *kinds* of mitochondria, in which morphology must play a minor and purely physical part.

In previous papers (Causey, 1925*a–d*) attention has been directed to the relation of the mitochondria to metabolic activities in the Protozoa. The part played by the rod-shaped mitochondria in anabolic activities has been discussed above. In *Endamoeba gingivalis* it was shown that the spherical mitochondria are associated with the formation of pseudopodia, there being a solation of the cytoplasm in the immediate vicinity of the spherical mitochondria in the region where the pseudopod is to form, and that the result is a very active Brownian movement of the mitochondria. The pseudopod forms during this period, and is always accompanied by the actively moving mitochondria. Upon the cessation of the Brownian movement of the spherical mitochondria, the pseudopod formation ceases. In *Noctiluca scintillans* the association of the spherical mitochondria with the cytoplasmic strands arising by streaming movement, and with the active flagellum, was shown, and was interpreted as evidence of the association of the spherical mitochondria with katabolic activities. *It is in protoplasmic movements that this katabolic activity is shown.*

The evidence at hand indicated that the neuromotor system is a fibrillar system for the coördination of locomotor organelles. As shown above, certain granules designated as blepharoplasts in flagellates and as basal granules in ciliates are a prominent feature of this system. The correlation of the spherical mitochondria with protoplasmic movements is an indication of how such a fibrillar system may arise without a change of function of the granules. In specimens of *Endamoeba gingivalis* in which the mitochondria have a definite spatial arrangement indicating a spiral organization, there appears to be a fibrillar connection between the mitochondria. To demonstrate this requires destaining carried to a very exact point. It is not a far step, morphologically, from such an arrangement to that shown in such ciliates as *Paramecium* where the same relation exists between the basal granules and the neuromotor fibrils. The same is true for the neuromotor system of a flagellate with its blepharoplasts and rhizoplasts, whether few in number as in the simpler forms, or very numerous as in the highly complex parasitic flagellates.

Although direct evidence for the derivation of the neuromotor granules from mitochondria is not available for protozoan forms, such evidence is to be had in metazoan tissues for a strictly comparable fibrillar system. Saguchi (1917) demonstrated in ciliated epithelial cells a typical neuromotor system in such cells, composed of the cilia, each with its basal granule and cytoplasmic strands which extend into the cytoplasm and become continuous with the cytoplasmic reticulum. This has been confirmed by Grave and Schmitt (1924), who have also investigated ciliated epithelial cells. Most significant in this connection, however, is Saguchi's account of the development of the ciliary apparatus. According to him there is at first an accumulation of mitochondria between the nucleus and the cell border. The mitochondria then migrate toward the cell border, and transform into rod-like bodies which become, eventually, the basal granules, and which give rise to the cilia. Not all the mitochondria are changed into basal granules; the remaining ones give rise to the accumulation of mitochondria so often demonstrated beneath a ciliated surface in both metazoan tissue and ciliates.

Although mitochondria cannot be demonstrated, ordinarily, with a fixative containing acetic acid, the granules of the neuromotor system are readily demonstrated with such a fixation. This does not negate the possibility of a mitochondrial origin of the granules. That mitochondria may become highly resistant to acetic acid has been indicated by the work of several investigators. Regaud (1910), for instance, reported for the mitochondria a progressive increase in resistance to acetic acid in the changes from spermatogonia to spermatozoa, which, it may be noted, involves, among other things, the development of motility. Nicholson (1916) has called attention to similar resistance to acetic acid on the part of the mitochondria in certain nerve cells. In the protozoa the presence of the so-called chromidia appears to be a case of badly preserved mitochondria (Gatenby, 1919).

The parabasal body, a spherical-shaped body in some flagellates and elongated in others, seems to be clearly of mitochondrial origin. This was suggested by Janicki (1915) and Swezy (1916), while Shipley (1916) indicated such an origin in his work on *T. lewisi*. Alexeieff (1917) has offered considerable evidence for such an interpretation, and demonstrated that such mitochondria or their derivative, the parabasal body, secrete glycogen. The latter was confirmed by Boeck

(1919). In *Leishmania brasiliensis* (Causey, 1925*b*), it was shown that the only apparent difference between the parabasal body and the spherical mitochondria, aside from the larger size of the former, was the resistance to acetic acid. The staining reactions, including staining with Janus green, are identical.

In this connection the recent work of Tanabe (1925) is of interest. Speaking of the parabasal body he says,

> This parabasal body seems to arise near the blepharoplast, but a connection between the two has never been found and a very narrow space always separates them. It is cylindrical, of various lengths and shapes and usually lies between the outer chromatic row and the nucleus; and ends after increasing in diameter near the distal end of this chromatic row. It appears distinctly in material fixed with Flemming's and chromic acid solutions. I have also found the parabasal body in *Trichomonas* from some species of termites (fixed in Flemming's solution without acetic acid and in chromic acid solution) but never in material fixed with Schaudinn's fluid. The fact that there appear to be individual variations in the parabasal body in parasites from different animals makes it a very interesting structure to study.

Similar differences in the stainability have already been reported by Kofoid and Swezy (1923) and Boeck (1917). The agreement of the various investigators points clearly to the variable resistance to acetic acid and staining reactions, typically that of mitochondria.

The axostyle seems to be of a similar origin. This, according to Kofoid and Swezy (1915), is "a powerful *motor* organ which comes into function when the animal is on a substrate, and doubtless plays an important part in the life of the organism in the mucous surface of the intestine" [italics mine]. The axostyle usually appears as a hyaline or weakly stained rod. According to Kirby (1924) it appears to arise as an outgrowth from the blepharoplast. The usual appearance of the structure in material fixed in Schaudinn's fluid indicates a lack of resistance to acetic acid similar to that evidenced by mitochondria in general. The hyaline appearance is to be interpreted as due to complete or nearly complete solution of the acetic acid, and the weakly stained appearance indicates a less complete dissolving by the acetic acid in the fixing fluid. Of interest in this connection is the axostyle present in *Tritrichomonas augusta* as described by Kofoid and Swezy (1915). Here the axostyle is an elongated rod in which are numerous spherical bodies which they term axostylar chromatin. It is probable that in this form the mitochondrial constituents of the axostyle are more resistant to acetic acid than are those found in other flagellates and, accordingly, are preserved sufficiently well to stain and show their structure.

In view of the above considerations, it seems probable that the granules of the neuromotor system—basal granules in ciliates and blepharoplasts in flagellates—may be considered as of mitochondrial origin, modified chemically to such an extent that they are resistant to acetic acid, and are still playing an active part in the katabolic processes as expressed by the motility of the organism.

SUMMARY

1. Mitochondria are demonstrated in the following ciliates: *Chaenia teres* Duj., *Microthorax sulcatus* Eng., *Colpoda saprophylla* Stokes, *Stylonychia pustulata* Ehr., *Euplotes patella* Ehr., and *Paramecium caudatum* Ehr.

2. The rod-shaped mitochondria are associated with the anabolic processes occurring in and about the food vacuoles.

3. The same relation of rod-shaped mitochondria to the food vacuoles is maintained throughout binary fission and conjugation, and is quite independent of either phenomena.

4. There is no evidence for the division of the mitochondria during the division of the micronucleus or of the cell proper. No evidence for division of the mitochondria at any time is found; they appear, instead, to arise *de novo*.

5. The granules of the neuromotor system in Protozoa appear to be derivatives of spherical mitochondria, modified chemically so as to be more resistant to acetic acid, but maintaining the katabolic functions shown by them in other Protozoa.

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EXPLANATION OF PLATES

All figures drawn with the aid of camera lucida from specimens fixed with osmic acid and stained with Regaud's modified haematoxylin.

PLATE 22

Fig. 1. Vegetative phase of *P. caudatum* showing the typical distribution of food vacuoles and the associated mitochondria. × 440.

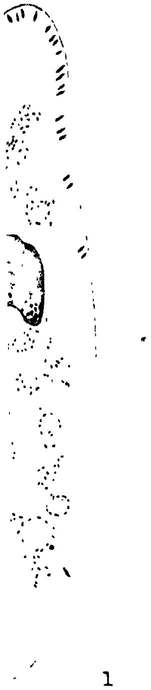
Fig. 2. Early exconjugant of *P. caudatum,* showing the association of the mitochondria with the food vacuoles, and lack of association with other structures. × 440.

Fig. 3. Late stage in binary fission of *P. caudatum.* Note the lack of any spatial arrangement of the mitochondria other than with regard to the food vacuoles. × 440.

Fig. 4. Early stage of conjugation in *P. caudatum.* The association of the mitochondria and the food vacuoles is identical with that in the vegetative organism. × 440.

Fig. 5. Exconjugant at end of the conjugation process in *P. caudatum,* just before cytopharynx is completely regenerated. The association of the mitochondria with the food vacuoles is still maintained. × 440.

Fig. 6. Early stage in binary fission of *P. caudatum.* No relationship to the division of the micronucleus is shown by the mitochondria. × 440.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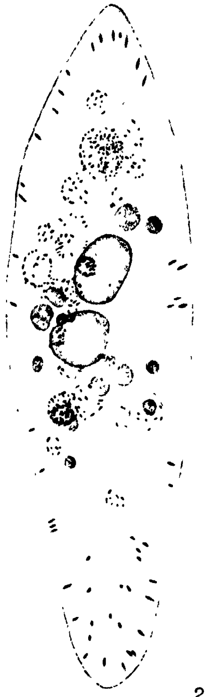
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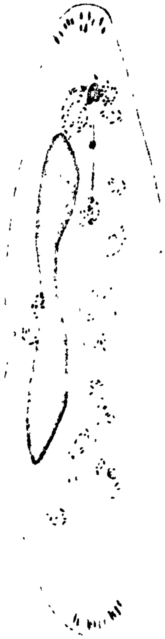
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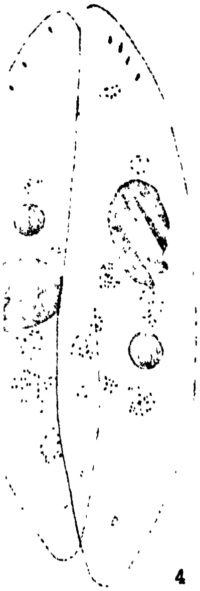
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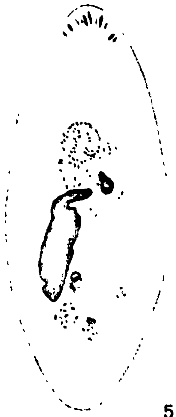
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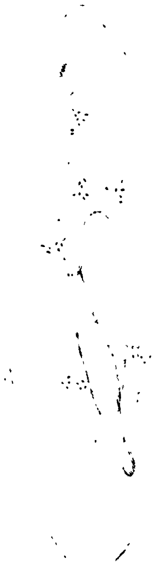
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PLATE 24

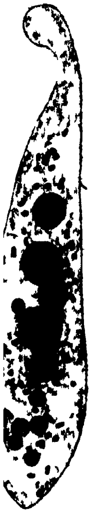
Fig. 13. *Chaenia teres* Duj. The mitochondria are distributed uniformly throughout the cytoplasm in the absence of food vacuoles. × 2170.

Fig. 14. *Microthorax sulcatus* Engelmann. The mitochondria appear to be almost exclusively in the ectoplasm. × 2170.

Fig. 15. *Colpoda saprophylla* Stokes. The mitochondria are distributed as in fig. 13. × 2170.

Fig. 16. *Stylonychia pustulata* Ehr. The specimen is unusual in that practically all of the mitochondria are spherical. The majority of specimens show typical rod-shaped mitochondria. × 1085.

Fig. 17. *Euplotes patella* Ehr. Specimen in very early phase of binary fission, showing typical rod-shaped mitochondria. × 1085.



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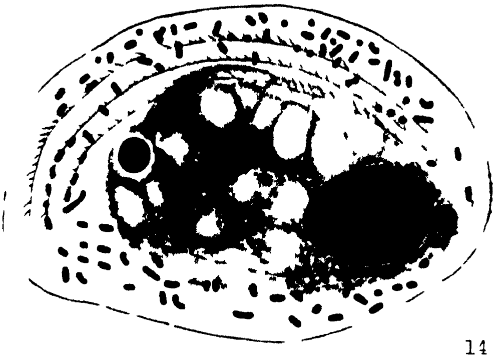

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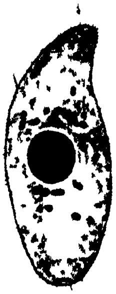

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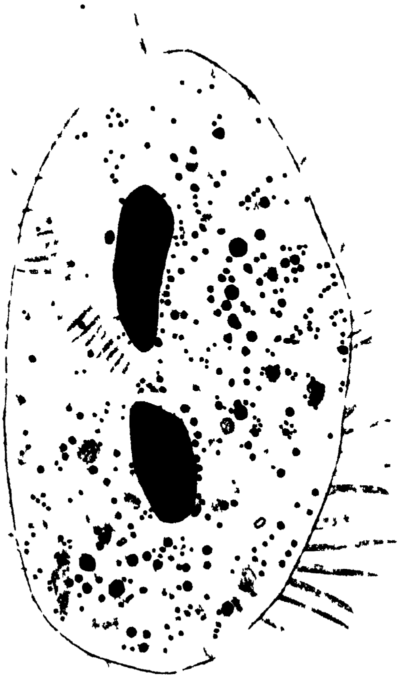

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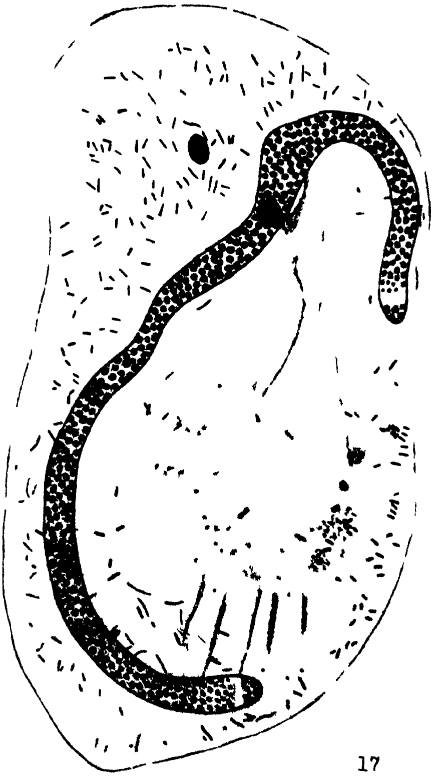

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STUDIES ON THE INGESTION OF LEUCOCYTES, AND ON MITOSIS, IN ENDAMOEBA GINGIVALIS

BY

HORACE J. CHILD

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CONTENTS

	PAGE
Introduction	251
Acknowledgments	252
Technique .	252
General morphology	252
Ingestion of leucocytes by amoebae	254
Ingestion of amoebae by leucocytes	258
Extrusion of siderophile food material	259
Mitosis	261
Resting stage	263
Prophase	266
Metaphase	269
Anaphase	270
Telophase	270
Summary	272
Literature cited	273
Explanation of plates	276

INTRODUCTION

The work presented in the following pages is the outgrowth of an investigation of the local distribution of *Endamoeba gingivalis* in the human mouth, undertaken by the author in collaboration with Professor Charles A. Kofoid, and Dr. George W. Simonton of the California Stomatological Research Group. The material for that investigation was obtained from cases of medium pyorrhoea in prisoners in the California State Prison at San Quentin. Its examination for the determination of the distribution of the amoeba disclosed also (1) certain interesting facts concerning the relation of this organism to the leucocytes commonly found in gingival exudate; (2) certain facts in addition to those observed by Kofoid and Swezy (1924) concerning its habit of egesting half-digested food bodies of leucocytic origin; and (3) a sufficient number of amoebae in various stages of mitosis to form a fairly complete picture of this process. These observations are herewith presented in the above-named order, preceded by a brief account of the general morphology of the amoeba.

ACKNOWLEDGMENTS

The author gratefully acknowledges his indebtedness to Professor Charles A. Kofoid, under whose direction this work has been carried on, and by whose counsel and criticism these first steps in his scientific research have been guided.

The author is also greatly indebted to Dr. Olive Swezy for courteous assistance in drawing, to Dr. F. V. Simonton for many valuable suggestions and criticisms, and to Dr. G. W. Simonton for assistance in collection of material.

For grants in support of this investigation acknowledgments are made to the Carnegie Corporation, the American Dental Association, the California State Dental Association, and the Associated Radiograph Laboratories.

TECHNIQUE

The amoebae upon which the following observations were made were present in smears of material removed with a dental scaler from surfaces of teeth in a typical case of active pyorrhoea. So far as possible, material was scraped from the entire subgingival surface of each side of each tooth, and therefore from all levels of the pockets. Smears were made by moistening the material in saliva and physiological salt solution, and spreading it very thin on slides. These were fixed in Bouin's fluid and stained in hot iron haematoxylin.

GENERAL MORPHOLOGY

Craig (1916) gave a brief account of the morphology and later Kofoid and Swezy (1924) made careful studies of the cytology of *Endamoeba gingivalis.* Although it is not the purpose of this paper to add to this general description, a brief supplemental account is given by way of introduction to the studies that follow.

The amoebae both in fresh smears and in fixed and stained preparations are commonly found in one of two forms. In the active form, endoplasm and clear ectoplasm are usually very distinct, and the ectoplasm is often extended to form one or two large, broadly rounded, clear pseudopodia, or many small, conical pseudopodia. The first are formed by the organism in locomotion; the second occur possibly in a period of recovery from shock (Kofoid and Swezy, 1924). In the rounded-up condition the amoeba is spherical, and often lacks a well defined region of ectoplasm. Such rounded-up amoebae have been interpreted by some investigators (Craig, 1915) to be encysted. Conclusive evidence of encystment is as yet lacking. In all normal amoebae in the resting stage, active or rounded, the cytoplasm is limited by a well defined pellicle of uniform thickness.

The diameter of *Endamoeba gingivalis* has been variously given as ranging from 6 to 60 microns. Twenty-five specimens in our fixed and stained material range from 7.5 to 35 microns in their greatest diameters. Amoebae smaller than 10 microns or greater than 25 microns are seldom encountered.

Details of structure are not evident in amoebae in fresh, unstained preparations. The endoplasm has a finely granular appearance, and normally contains numerous vacuoles of various sizes, in some of which food inclusions are faintly visible. The nucleus is seldom visible in unstained specimens. The ectoplasm and pseudopodia, when present, are very clear. Endoplasm and ectoplasm are sharply delimited from each other, and the contrast between them is striking. In optical section at the greatest circumference the limiting pellicle of the ectoplasm is an impressively dark, sharp line.

In fixed amoebae stained with iron haematoxylin the ectoplasm is usually finely granular or faintly alveolar in appearance. The limiting pellicle stains darkly and uniformly. Ectoplasm and endoplasm are not so sharply delimited from each other as in unstained material. The endoplasm is granular or alveolar. It usually contains and is often packed with spherical or subspherical vacuoles ranging from ten microns in diameter down to a very minute size. These contain a clear fluid in which are floated in the larger vacuoles remains of ingested leucocytes (pl. 25, fig. 3), or of leucocytic nuclei devoid of cytoplasm (same figure, smaller inclusions). Such nuclear food bodies, if recently ingested and only partially digested, are ovoid in shape and stain homogeneously black in iron haematoxylin. Their contrast with the clear fluid surrounding them in the vacuoles is striking. As digestion proceeds the food bodies stain irregularly, and fragmentation takes place. Finally they lose their stainability, and appear as gray, shapeless masses which ultimately disappear, leaving clear, fluid-filled vacuoles. These decrease in size as their contents are assimilated, so that an amoeba usually contains vacuoles of greatly varying sizes.

Some amoebae contain, almost exclusively, food bodies of bacterial origin. These may range from minute diplococci (pl. 29, fig. 37) and small bacilli (pl. 28, figs. 22, 23, 29), to large fusiform rods (pl. 29, fig. 44) which may distort the amoeba, and long alga-like filaments (pl. 29, fig. 46) several times the length of the amoeba and necessarily coiled within it.

INGESTION OF LEUCOCYTES BY AMOEBAE

Prowazek (1904) asserts that "*Entamoeba buccalis*" uses leucocytes as food, but that the nuclear remains seem not to be digested; at least, that they are always cast out. With no figures, and no description of the food bodies he observed, it is impossible to ascertain whether his statements are based on observations of leucocytes actually ingested whole, or on observations of ingested "salivary corpuscles," degenerating leucocytic nuclei devoid of cytoplasm. In either case, he evidently noted the extrusion of viscous nuclear remains in certain cases, and failed to note the progressive steps, in most instances, in the digestion of these food bodies. He concludes then that they are the nuclei of ingested leucocytes and that they are expelled after the digestion of their cytoplasm.

Leyden and Loewenthal (1905) state that the food seems to consist mainly of leucocytes, with the remains of which the amoebae are often filled. From this brief statement and their single figure it seems probable that they observed only ingested nuclei devoid of cytoplasm. They do not note the presence of any trace of cytoplasm surrounding such nuclei. These may therefore have been nuclei of leucocytes ingested whole, whose cytoplasm had been digested, or more probably, merely salivary corpuscles, nuclei of degenerating leucocytes devoid of cytoplasm when ingested.

Craig (1916) states that there are numerous ingested bodies, most common of which are nuclei of leucocytes, bacteria, and round or oval bodies, probably of protozoan nature. His description of these last is confusing: ". . . parasites filled with oval or round bodies, stained black, and surrounded by a well marked unstained area. These bodies are evidently contained within vacuoles." These may have been black-stained leucocytic nuclei devoid of cytoplasm, contained in clear vacuoles. But Craig's description gives the impression of an ingested body consisting of a clear outer zone surrounding round or oval bodies, and the whole contained within a vacuole.

This suggests ingested, partly digested leucocytes (pl. 25, figs. 3, 4) but is not conclusive.

Smith and Barrett (1915) say that the digestion vacuoles contain globular detritus of leucocytic nuclei and red blood cells along with bacteria. Doflein (1916) states that the food seems to consist mainly of leucocytes and bacteria. Hartmann and Schilling (1917) say that the food consists of the bacteria of the mouth, but chiefly of leucocytes and leucocytic remains or salivary corpuscles.

Nowlin (1917*b*) believes that *E. gingivalis* does not ingest leucocytes, but takes its fluids by osmosis, leaving the leucocytes intact. She draws this conclusion because she is unable to find more than one body in a vacuole, while leucocytes have two or three nuclei; she is unable to find any granular area around the inclusions, as would be the case if the cytoplasm of a leucocyte were ingested; she has observed amoebae surrounding leucocytes, but never ingesting them. Forgetting temporarily, it would seem, that the digestive process would normally liquefy food bodies, she cannot conceive the leucocytic origin of the inclusions in this amoeba because they show "never any resemblance to leucocytes" and appear "more as globules of fluid."

Goodey and Wellings (1917) have made a careful study of the ingesta of *E. gingivalis*, realizing that upon the determination of whether these were the products of leucocytes ingested whole "depended to a large extent the answer to the question whether or no *E. gingivalis* is a true parasite of pathogenic importance." They found in salivary smears large numbers of "salivary corpuscles," or disintegrating leucocytes. These consisted of single nuclei or groups of lobes of nuclei, with attached fragments of cytoplasm or entirely naked. In size, shape, and stainability these gave an exact picture of the ingested bodies found in the amoeba. Goodey and Wellings searched in vain for evidence of polymorphonuclear leucocytes ingested whole, concluded that the amoeba feeds upon nuclei of disintegrating leucocytes devoid of cytoplasm, and that, far from having any pathogenic significance, it may rather be considered a useful organism.

Kofoid and Swezy (1924) find "as a rule only nuclei or their disintegration products in the food vacuoles, not normal leucocytes with cytoplasm" with only occasional evidence of a cytoplasmic film around the nucleus. "This may be because of the prompt digestion of the cytoplasm, or of the ingestion of naked nuclei from the cellular detritus which abounds in the smears."

Several specimens in the stained preparations of the present investigation demonstrate conclusively that *Endamoeba gingivalis* does ingest both "salivary corpuscles" and not infrequently whole, normal leucocytes.

In evidence of ingestion of naked nuclei there is an amoeba (pl. 25, fig. 9) containing in a typical food vacuole a leucocytic nucleus or single lobe of one, quite devoid of cytoplasm. Its diameter (3.5 microns) is that of a large lobe of a polymorphic nucleus. Its structure is that of such nuclei stained in iron haematoxylin (cf. nuclei of leucocytes in pl. 25, figs. 8, 10): large, roughly conical masses of peripheral chromatin whose attenuated apices extend to the irregular central mass of chromatin. In size and ovoid shape this ingested body corresponds with the average solid black-staining inclusions found in amoebae. Very evidently it is identical with these, except that the disintegration of its cytoplasm and the ingestion of the naked nucleus by the amoeba have taken place so recently that its structure and stainability have not been altered by degeneration or digestion.

Such nuclei, naked or with a trace of cytoplasm, are numerous in many smears in these preparations. Depending upon the recency of the death of the leucocytes from which they are derived, and the degree of their degeneration, these may stain so as to show their normal structure as in figure 9, or they may stain solid black as in most of the figures of Goodey and Wellings (1917, pl. 21, figs. 13*a*–13*c*, 14*a*–14*m*), or there may be intermediate stages in which the structure is only partially recognizable.

When such nuclei with structure intact are ingested by amoebae their digestion and liquefaction must be very rapid, as evidenced by the infrequency of structurally recognizable ingested nuclei. In many cases no doubt disintegration is well advanced before ingestion takes place. The digestion of these bodies may be followed through stages in which the regular contour and homogeneous stainability are lost; through fragmentation, with entire loss of chromatin-staining properties; and ultimate disappearance, followed by decrease in size of the vacuoles.

In evidence of the ingestion of whole leucocytes there are two amoebae (pl. 25, figs. 1, 2) in which fixation occurred while the actual process of ingestion was in progress; and besides these, several amoebae (pl. 25, figs. 3–7) containing leucocytes in various stages of digestion. The lobed character of the nuclei of the leucocytes in these cases places them among the polymorphonuclear leucocytes or the eosinophiles. Beyond this they cannot be differentiated in the iron haematoxylin stain.

In the first of these cases (pl. 25, fig. 1) a small amoeba is engulfing a leucocyte of about its own size. The cytoplasm of the amoeba is extended in three small pseudopodia (with possibly a fourth on the other side) which are surrounding the leucocyte, closely applied to its surface. The surface of the amoeba between the pseudopodia is cupped around the intruding end of the leucocyte. Both amoeba and leucocyte are slightly elongated in the direction of their common axis, and the cytoplasm of the leucocyte is constricted at the end which is being drawn into the amoeba. The cytoplasm of the leucocyte has a coarsely reticular appearance, which is normal for leucocytes stained with iron haematoxylin. The nuclei are typical (slightly overstained). The contrast between the cytoplasm and nuclei of the leucocyte and those of the amoeba is marked.

In the second instance (pl. 25, fig. 2) the amoeba has almost completed the ingestion of a leucocyte. The portion already engulfed is contained in a very large vacuole, and is surrounded by a clear fluid. A portion of the cytoplasm as yet uningested protrudes through an opening in the vacuole and cell wall. The size of the opening necessitates a decided constriction of the leucocyte, suggesting that the portion which flares outside the amoeba is perhaps to be pinched off. This flaring cytoplasm is typically, coarsely reticular. Within the vacuole, the leucocyte is rapidly being digested, so that the lobed nucleus stains solid black and the cytoplasm stains darkly. In the cytoplasm most recently ingested, a trace of the normal reticular structure remains.

The amoeba represented in figure 3 has completely ingested a leucocyte, and digestion and disintegration are progressing rapidly. The black-staining lobes of the nucleus are embedded in a thick film of disintegrating cytoplasm, which stains darkly. The containing vacuole is very large.

As the digestive process continues, the cytoplasmic mass decreases in volume (pl. 25, fig. 4) and finally disappears entirely, leaving only the black lobes of the nucleus (fig. 5). The containing vacuole is still large compared with those containing ingested "salivary corpuscles." As the nucleus undergoes liquefaction its lobes lose their ovoid shape (pl. 25, fig. 6) and become closely applied to each other, forming a single spherical mass. They finally become quite fused (fig. 7) so that their boundaries are traced with difficulty.

As further reduction in size of the fused lobes takes place, this mass becomes indistinguishable from ingested "salivary corpuscles." It is therefore certain that a considerable number of the normal, black, round or oval food bodies of the amoeba represent late stages in the digestion of leucocytes ingested whole. However, the relative infrequency of food bodies definitely recognizable as whole leucocytes indicates that "salivary corpuscles" probably constitute a greater part of the food of the amoeba than do normal leucocytes.

Goodey and Wellings (1917), failing to find evidences of ingestion of whole leucocytes, made the sweeping conclusion that these were not ingested by *E. gingivalis*, and upon this conclusion built their argument against the pathogenicity of the organism. Their evidence, however, is negative evidence. The observations here presented demonstrate that *E. gingivalis* does ingest whole leucocytes as well as "salivary corpuscles" and bacteria, and show that the conclusions of Goodey and Wellings were based on insufficient observations.

The literature on both sides of the controversy over the pathogenicity of *E. gingivalis* contains much that is inaccurate, with sweeping conclusions drawn from inconclusive results. Although today the organism is generally considered to be a harmless commensal, there has been little if any critical experimentation to establish definitely its relation to diseased conditions in the mouth, and no successful treatment for the infestation is known. The fact that the amoeba destroys leucocytes to an extent as yet undetermined, suggests that it may not be entirely harmless, and indicates that the subject of its pathogenicity is not a closed one.

INGESTION OF AMOEBAE BY LEUCOCYTES

Leucocytes, by uniting with other leucocytes to form a giant cell, may surround an amoeba and enclose it in a vacuole. This phenomenon is demonstrated by two stained specimens. In the first (pl. 25, fig. 8), three leucocytes with polymorphic nuclei are actively surrounding an amoeba. One leucocyte is below the amoeba as figured, and two above. Two of the three leucocytes are spread out over and closely applied to the surface of the amoeba. At least two of the leucocytes are beginning to fuse, so that their opposed boundaries are not distinguishable. The amoeba is still active, as evidenced by two small, typical pseudopodia of the conical type.

The second example (pl. 25, fig. 10) is evidently a later picture in the same process. Three leucocytes with polymorphic nuclei have fused, forming a giant cell completely surrounding an amoeba. The captured organism is contained in a large vacuole common to the bodies of the leucocytes, and is surrounded within the vacuole by a clear fluid. The amoeba is inactivated, as shown by absence of pseudopodia, and by an abnormally heavy pellicle. It shows as yet, however, no marked signs of degeneration. The leucocytes are actually fused, so that their boundaries are marked only by a superficial lobing of the giant cell.

The conclusion of this interesting process has not been observed. If, as we might expect, the amoeba is killed and the whole mass disintegrates, it is probable that the occurrence is more common than is indicated by the infrequent examples demonstrable in smears.

Bond (1924) has described a similar fusion of two, three or more leucocytes which had ingested sheep red cells. Injection of sterile paraffin, sand, or other bodies into rabbits is known to produce a similar reaction on the part of rabbit leucocytes. It is of interest here merely as a reaction of the leucocytes toward foreign bodies, and has no bearing upon the pathogenicity of the amoeba.

EXTRUSION OF SIDEROPHILE FOOD MATERIAL

Instances of amoebae with extrusions of viscous, deep-staining, siderophile food material of leucocytic origin as described by Kofoid and Swezy (1924) are numerous in stained preparations (pl. 26). As noted in an earlier section, Prowazek (1904) seems to have observed these, but did not figure them, and concluded that nuclear remains of ingested leucocytes were always egested. Successive stages in the digestion and absorption of such remains, however, show that they are normally utilized as food, and that their expulsion is exceptional.

In a very few examples noted, external pressure exerted in the smearing process seems to have caused the expulsion. In such a case the amoeba is distorted, the vacuole from which the extrusion arises is usually near the surface, and the cytoplasm in its vicinity is flattened, showing signs of pressure.

The normal appearance of most amoebae with extrusions of food material indicates, however, that the expulsion is a normal process, produced by internal pressure. In these cases there is no distortion, and the emptying vacuole may be some distance from the surface of the amoeba, so that the strand extends through some microns of cytoplasm between vacuole and pellicle (pl. 26, figs. 14, 17). The portion of the strand thus enclosed is contained in a canal which is continuous with the vacuole from which the extrusion arises, and which contains, surrounding the strand, the usual clear fluid of the vacuole (fig. 14). As described by Kofoid and Swezy (1924), in well stained specimens a ring of thickened pellicle is demonstrable at the point at which the strand traverses the pellicle.

This siderophile material is evidently extremely viscous, and may be drawn out in long strands. One amoeba, 12 microns in diameter, rounded and seemingly moribund, has a strand drawn out to a total length of 110 microns. Another amoeba (pl. 26, fig. 17), 7 by 9 microns, has an extrusion 67 microns long. In most cases the strand does not taper regularly from the amoeba to its tip, but may be enlarged, forming a hump just outside the amoeba, tapering gradually from this to a fine, attenuated strand (fig. 12, upper strand; fig. 17); or it may terminate in a flare (fig. 12, lower strand); or occasionally in a knob (fig. 14). Only occasionally does a strand taper smoothly from the amoeba to the tip of its free end. In five instances recorded, there are extrusions from two vacuoles in a single amoeba.

Not infrequently two amoebae are found connected by a strand of siderophile material (pl. 26, figs. 11, 15). This may be the result of fusion of the free ends of extrusions from both amoebae (Kofoid and Swezy, 1924) but more often gives the impression of being the result of ingestion by one amoeba of the free end of a strand egested by the other. The reasons for so assuming are twofold. (1) Connecting strands do not have irregular humps or flares between the amoebae connected, such as would be present if two strands had fused. Rather, they are smooth and finely attenuated (pl. 26, fig. 11). The only enlargements are usually terminal, and these are contained in vacuoles in the connected amoebae. In only one out of eleven cases was there a marked irregularity in the strand between the amoebae. (2) Two amoebae thus connected are frequently (7 out of 11 cases) closely applied to each other at the points at which the strand traverses their pellicles (fig. 15), so that this is within their bodies throughout its entire length. To have become thus closely applied to each other, one or both of the amoebae must have ingested whatever portion of the extrusion may have been free originally. That the egested food of one amoeba should be acceptable to another, suggests that the expulsion process may be the result of other stimuli than undesirable food.

In one case (pl. 26, fig. 16) two amoebae are connected by two strands. In another instance (pl. 27, fig. 20) each of two amoebae has a connection with a third amoeba. And in another case (pl. 26, fig. 13) a polymorphonuclear leucocyte has ingested the flaring free end of a siderophile extrusion.

MITOSIS

Early investigators considered that nuclear division in *Endamoeba gingivalis* was accomplished by a process of simple mitosis or even amitosis. Prowazek (1904) says that the karyosome of "*E. buccalis*" divides mitotically, but that the surrounding achromatinic network with its chromatin granules divides amitotically. He also describes a spindle stretched between granular poles, and bearing an equatorial plate. This suggests the metaphases figured in plates 28 and 29 of this paper.

Leyden and Loewenthal (1905) confirm Prowazek's observations on the division of the karyosome. They find a "*stabförmige*" spindle in the middle of which deep-staining particles form an equatorial plate. These are undoubtedly clustered chromosomes in the metaphase. They observe also what they consider a second type of nuclear division, in which an elongated nucleus (their fig. 7) has at either end a mass of substance staining black in iron haematoxylin. In this they figure a ring-shaped "*Binnenkörper*" which is not interpretable. They also figure several scattered granules within the nucleus, not mentioned in their text, but which suggest chromosomes. The whole picture, though inconclusive, suggests the anaphase of mitosis, with an elongated nucleus, heavy polar caps, and well separated chromosomes.

Craig (1916) briefly describes a process of simple mitosis in which the karyosome divides into two parts, connected by a delicate thread of chromatic material. Rarely, he says, a more or less distinct nuclear spindle may be observed. The nucleus elongates and constricts in the center. It seems probable that the dividing structure which he interprets as the karyosome is in reality a close-massed cluster of chromosomes in the metaphase. The delicate thread of chromatic material suggests the intradesmose. His binucleate amoebae, the membranes of whose nuclei are thin and without any demonstrable peripheral chromatin, seem to be telophases as represented in figure 39, plate 29 of this paper.

Goodey and Wellings (1917) find one binucleate amoeba and a late stage in the fission of an amoeba. They also note that in several preparations the karyosome appears to be absent, and that "the space within the nuclear membrane is taken up with a network of linin fibres on which small granules of chromatin are irregularly disposed." This definitely suggests the early prophases represented in figures 22 and 23, plate 28, of this paper, in which karyosomes are absent, and chromosomes are forming on the spoke radii.

Nowlin (1917*a*) believes, contrary to Craig, that the mitotic process in *E. gingivalis* is a complex one. She notes the disappearance of the karyosome in the early stages. She figures a metaphase and an anaphase with spindles and chromosomes, but makes the very confusing suggestion that four large blobs on the periphery may correspond to chromosomes in higher animals. Her figures are not convincing. She writes that the greater prevalence of prophases in her smears indicates that this stage is of longer duration than are the others, a fact which is borne out by observations in the present investigation.

Several factors have no doubt contributed to this confusion. The nucleus and chromosomes in mitosis are extremely small. Also, the nucleus in mitosis does not show any marked elongation until the anaphase. Consequently, in superficial examination prophases and metaphases are often mistaken for normal resting stages. Except in specimens differentiated with utmost care, the spindle of the metaphase is not always readily demonstrable. Particularly in amoebae insufficiently destained the central cluster of chromosomes in the metaphase and late prophases is likely to be mistaken for the normal central karyosome of the resting stage. Late stages in the process seem not to be abundant.

In smears fixed in Bouin's fluid and properly stained in iron haematoxylin, optimum optical equipment has revealed a process of true mitosis in *Endamoeba gingivalis*, similar in all essential respects to the mitotic process in the other Amoebina. There is no evidence of a simple process of amitosis as reported by earlier investigators.

There is no evidence of multiple fission as reported by Nowlin (1917*a*). There is no evidence of conjugation, as reported by Craig (1916) and as "suspected" by Nowlin (1917*a*).

RESTING STAGE

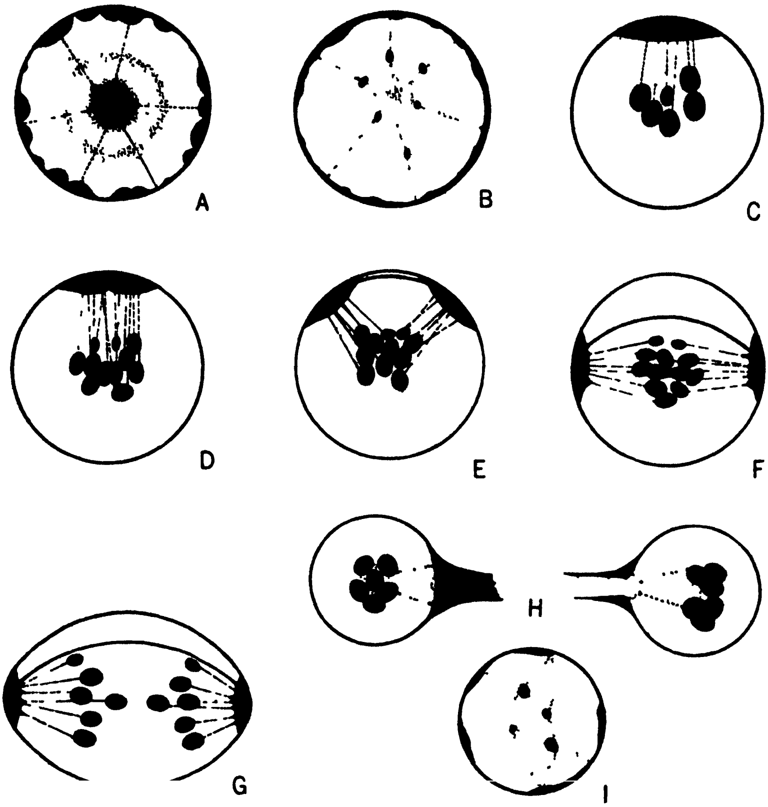
Figure A; plate 28, figure 21

In the resting stage the nucleus of *Endamoeba gingivalis* properly stained in iron haematoxylin appears as a spherical or subspherical vesicular body. Pressure of food vacuoles may often very much distort it (pl. 25, fig. 2; pl. 29, fig. 44). In specimens in which it is not thus crowded, it may vary from almost perfect sphericity (pl. 25, fig. 4) to a condition of more or less irregularity (pl. 26, fig. 13, and 15, lower amoeba).

The diameters of twenty nuclei measured, of twenty amoebae from two persons, ranged from 1.87 microns to 4.2 microns, averaging 3.2 microns. Kofoid and Swezy (1924) find nuclei with diameters ranging from 1.8 to 5.6 microns in 75 amoebae from three different persons. These average 2.93 microns for 25 amoebae from one case, 3.34 microns for 25 amoebae from a second case, and 4.01 microns for 25 in the third case. In the third case, where there is a history of pyorrhoea for 30 years, diameters of nuclei average 30 per cent greater than those of the first case, in which there is no history of pyorrhoea. Other investigators have described the nucleus as ranging from 1.5 to 6 microns.

The nuclear membrane, a thin film of uniform thickness, is demonstrable in the resting stage, only in heavily destained nuclei. In nuclei more heavily stained, it is obscured by the peripheral chromatin, which appears in beads closely applied to its inner surface. Seen in optical section in heavily destained nuclei, these appear as more or less discrete beads, their inner surfaces rounded, and outer surfaces flattened against the nuclear membrane. In heavily stained nuclei these beads lose their discreteness, so that the membrane and peripheral chromatin together form a heavy ring, in optical section, gently lobed on its inner surface. There is often some irregularity in the distribution of the peripheral chromatin, so that it may appear in one, two, or three masses on the circumference in optical section (pl. 26, fig. 11, upper amoeba; fig. 14; fig. 16, both amoebae).

In heavily destained nuclei the central or slightly subcentral karyosome appears to consist of from one to several large, solid, black-



Figures A–I. Nuclei of *Endamoeba gingivalis* in Mitosis.

Fig. A. Resting stage, showing karyosome, granular halo, spoke radii, and peripheral chromatin.

Fig. B. Early prophase, with six chromosomes appearing as granules on the spoke radii. Peripheral chromatin is disappearing.

Fig. C. Later prophase. Six undivided chromosomes with fibers extending to the centrosome or large peripheral mass of chromatin. Except this mass, the nuclear membrane is devoid of chromatin.

Fig. D. Later prophase. Five chromosomes have divided. One is undivided. The centrosome is beginning to divide.

Fig. E. Late prophase. The centrosome has divided, and the daughter centrosomes are separating along a meridian of the sphere, drawing out between them a connecting strand or intradesmose.

Fig. F. Metaphase. The centrosomes are diametrically opposed. They are connected meridionally by the intradesmose; diametrically by the spindle on which the chromosomes are clustered. The sixth parent chromosome is dividing.

Fig. G. Anaphase. The chromosomes are migrating along the spindle fibers toward the polar masses.

Fig. H. Telophase. The nucleus has constricted and divided. The breaks in the nuclear membranes are not yet closed. The chromosomes are closely grouped in each daughter nucleus. Each polar cap has diminished in size, and chromatin substance has become concentrated in the regions of the breaks in the nuclear membranes. Vestiges of spindle fibers connect the chromosomes with these masses.

Fig. I. Single daughter nucleus in late telophase. The chromosomes are disappearing. The peripheral chromatin and spoke radii are reappearing.

staining granules, usually embedded in a cloud of fine, lighter-staining granules (pl. 25, fig. 5; pl. 28, fig. 21). In specimens more heavily stained and possibly actually in a different stage of nuclear organization, the larger central granules are not demonstrable. Under such conditions the whole mass stains darkly, and its surface has a lobed appearance (pl. 29, fig. 43) or may be drawn out in blunt or sharp points (fig. A; pl. 25, fig. 2; pl. 26, fig. 11).

From these points or perhaps ultimately from the large granules within the mass, fine, filamentous spoke radii, often six in optical section, extend to the periphery of the nucleus, where they seem usually to lose themselves in beads of peripheral chromatin.

Between the karyosome and the periphery of the nucleus, separated from the periphery by a clear space which Kofoid and Swezy (1924) have called the intermediate zone, is a granular halo (fig. A; pl. 28, fig. 21). It appears in optical section as an irregular ring of fine granules, following only roughly the contour of the nuclear membrane, often polygonal rather than rounded, projecting angularly at the points at which the spoke radii traverse it. Its outer margin is sharply delimited from the surrounding, clear intermediate zone, but its inner margin may be seen in good preparations to merge by a region of less deeply staining, more dispersed, fine granules into the dense cloud surrounding the karyosomal granules.

In the resting stage the cytoplasm usually contains and is often packed with food vacuoles containing black-staining remains of leucocytes and "salivary corpuscles." Recently ingested and only partially liquefied nuclei stain solid black and are contained in large, clear, liquid-filled vacuoles which may press and distort the nucleus of the amoeba. The food bodies may partially or entirely obscure the nucleus of the amoeba. In later stages of digestion, the food bodies undergo fragmentation, the fragments lose their stainability and disappear, leaving clear vacuoles. These decrease in size, so that vacuoles in an amoeba may range from ten microns down to a minute size. It may be these smallest which give the cytoplasm its alveolar appearance. Other vacuoles contain ingested bacteria, but usually not in large numbers in amoebae containing leucocytic inclusions.

In the normal amoeba whose nucleus is in the resting stage, the endoplasm is often though not always sharply delimited from the ectoplasm. The latter is often extended to form large, clear pseudopodia. The ectoplasm is limited by a pellicle, usually of uniform thickness and stainability.

PROPHASE

Figures B–E; plate 28, figures 22–30

The earliest stages in the transformation of the nucleus of *Endamoeba gingivalis* from the resting stage into the prophase have not been found. In the earliest stage available (pl. 28, fig. 22) the karyosome as such has disappeared. By analogy with the findings of Swezy (1922) in *E. coli* and of Kofoid and Swezy (1925) in *E. dysenteriae,* we might assume that the karyosomal centrosome had migrated to the periphery and had divided to form two daughter centrosomes. However, there is no evidence that this is the case in *E. gingivalis,* while there is evidence that the division of the centrosome takes place considerably later than these first stages. If we assume that the centrosome is a small granule contained in the resting stage within the karyosome, it is possible that this migrates to the periphery in the very early prophase, and becomes obscured in the peripheral chromatin. Its division, however, evidently does not occur until later.

The chromosomes first appear as six small granules on the spoke radii, and more or less scattered within the sphere of the nucleus (fig. B; pl. 28, fig. 22). They tend to be slightly elongated, and to lie with their long axes along the spoke radii, which converge toward the center of the nucleus. The spoke radii are not all traceable to the center, but as some of them are, it is probable that the central, converging ends of the others are obscured by their fineness, and by imperfect staining. There is a fairly definite granular halo in the normal position. This halo, together with the spoke radii and other granules scattered in the sphere, gives somewhat the appearance of a fine network.

The peripheral chromatin appears in optical section as a heavy band whose inner margin is gently lobed. There seems to be little if any decrease as yet in the actual amount of peripheral chromatin, though this is a matter difficult of determination.

The chromosomes migrate centrally along the spoke radii (pl. 28, fig. 23) so as to become grouped in the center of the sphere (figs. 24, 25). During this migration they increase in size and become definitely ovoid or bluntly rod-shaped, black-staining bodies. Simultaneously with the migration and growth of the chromosomes there is a gradual disappearance of the peripheral chromatin, so that it seems probable that it contributes in part at least to their substance. The granular halo partly disappears, and may also contribute to chromosome formation. The peripheral chromatin which remains has a beaded appearance, and the beads tend to be grouped in two or three masses in optical section. The chromosomes are connected with the beads of peripheral chromatin by six fine spoke radii.

In figure 25, plate 28, there is a slight suggestion of bunching of the peripheral chromatin on one side of the nucleus (lower side in figure). In the next available stage (pl. 28, fig. 26) the nuclear membrane is devoid of chromatin except for one large mass. To this mass converge the six fibres, one from each chromosome. It would seem from the foregoing that the beads of peripheral chromatin remaining after formation of the chromosomes, migrate from their positions about the periphery, collecting in a single mass on one side. As they do so, they evidently carry the spoke radii with them so that these converge into the mass, connecting the chromosomes with it.

If we assume that a centrosomal granule contained within the karyosome during the resting stage migrated to the periphery in the earliest stages of the prophase as suggested above, we can conceive this mass of chromatin as surrounding the now peripherally located centrosome. As stated in an earlier paragraph, there is no evidence of such an occurrence in *E. gingivalis*, though the earliest stages are lacking in the series. Such a centrosome if present does not divide in the early prophase as in *E. dysenteriae*, but in the late prophase, after several of the chromosomes have divided. The single piece of evidence for such a granule in *E gingivalis* occurs in an early anaphase (pl. 29, fig. 35). Here a granule appears between the nuclear membrane and one polar cap, surrounded by a clear space, and suggesting a daughter centrosome within or behind the cap.

After the chromosomes are fully formed and the remaining peripheral chromatin has collected in a single peripheral mass, gathering the spoke radii so that they converge into this mass or centrosome and connect the chromosomes with it, each chromosome divides forming two daughter chromosomes. Each chromosomal rod seems to split lengthwise (pl. 28, fig. 28, chromosomes nearest centrosome). They do not divide synchronously, so that prophases are found with varying numbers of chromosomes. In figure 27, plate 28, three have divided and three are undivided. In figure 28 five have divided and one is undivided, so that there is a total of eleven. The time of the division of the fourth and fifth chromosomes with respect to the steps that follow is subject to some variation, and the sixth chromosome usually does not divide until the late metaphase. A characteristic number of chromosomes appearing in these late stages of the prophase is therefore 9 or 11, and for the early metaphase, 11. In general, the long axes of the chromosomes may be said to lie in the direction of the axis of the cone of fibers connecting them with the centrosomal mass. This is subject to some variation, however, so that in some cases no definite orientation exists until the anaphase.

While the chromosomes are dividing, a notch appears in the centrosomal mass, giving it a bilobed appearance (pl. 28, fig. 28). The whole mass, possibly activated by the division of a centrosomal granule within it, divides, and the two daughter centrosomes move apart (figs. 29, 30), each carrying a set of spindle fibers with it.

As the centrosomes divide there is drawn out between them a fine strand or intradesmose, corresponding with the similar structure found by Kofoid and Swezy (1921) in *Councilmania lafleuri,* and named by them. A similar structure occurs also in *E. coli* (Swezy, 1922), and in *E. dysenteriae* (Kofoid and Swezy, 1925). It appears in figures 29 and 30, plate 28, as a dark-staining fiber stretched in a meridional position between the separating polar masses and closely applied to the inside of the nuclear membrane.

The polar masses migrate around the periphery of the nucleus until they become diametrically opposed. Between them is the spindle, formed of fibers which they have carried with them, bearing midway between its poles the chromosomes which usually number 11 at this stage, five split and one not yet divided. With its polar masses diametrically opposed, the nucleus is in the metaphase.

A striking feature of most amoebae in the prophase of mitosis is the absence of typical leucocytic food inclusions. In a few cases observed, partially digested remains of such food bodies persist in the prophase (pl. 28, fig. 25); in another case an amoeba in the metaphase contains leucocytic inclusions in an advanced stage of digestion (pl. 29, fig. 33); and an amoeba whose nucleus is in the anaphase contains one typical leucocytic food body and another in an advanced stage of digestion (pl. 29, fig. 36). For the most part, however, the cytoplasm of amoebae in division stages contains only fluid-filled vacuoles in which the leucocytic nuclei have been completely or almost completely digested, so that the vacuoles appear nearly or entirely clear. In amoebae in the prophase these are large (pl. 28, figs. 24, 26, 28), indicating a recent completion of the digestion of the food bodies.

In later stages of division, the vacuoles tend to be smaller, so that the cytoplasm sometimes presents a very finely alveolar appearance.

A notable difference between the persistence of ingested bacteria and that of leucocytic nuclei is demonstrated in most amoebae in mitosis. Amoebae in almost all stages of mitosis contain some form or forms of ingested bacteria. This may possibly mean that amoebae ingest bacteria after the initiation of mitosis, but more probably means that the digestion of bacteria is slower than that of leucocytic food.

These facts indicate that ingestion of food stops prior to the inception of mitosis, and that digestion of food of leucocytic origin is usually completed during the early stages of division, while bacterial inclusions persist somewhat longer. There is however little or no evidence of a glycogen-chromatoidal body cycle as in encysted forms of *Endamoeba* and *Councilmania*.

Amoebae in mitosis tend to round up in the late prophase and thereafter. These rounded forms often put forth small pseudopodia (pl. 28, figs. 29, 31; pl. 29, fig. 35) which are not of the broad type used in locomotion. A further feature of amoebae in mitosis is the lessened thickness and distinctness of the pellicle. This is subject to some variation, but in some cases becomes so thin as to be barely discernible (pl. 28, fig. 29; pl. 29, fig. 39).

METAPHASE

Figure F; plate 28, figure 32; plate 29, figures 33, 34

In the metaphase the polar masses have become separated a maximum distance from each other within the spherical nucleus, so as to be diametrically opposed. The nucleus may now become slightly elongated along the polar axis. The polar caps appear as broad cups with irregularly projecting margins, and convexly fitted into the rounded or broadly conical ends of the nucleus. Stretched between the poles is the spindle, bearing the chromosomes in its equatorial region. Generally, the polar ends of the spindle fibers seem to be attached to or to disappear into the projections on the margins of the polar caps.

In the early metaphase the chromosomes usually number eleven, the division of the sixth parent chromosome taking place slightly later than that of the other five. The chromosomes in this stage are ovoid or bluntly rod-shaped, and of uniform size except one pair which is markedly smaller than the others in most instances. These smaller chromosomes often appear slightly apart from the rest of the cluster (pl. 28, fig. 32; pl. 29, fig. 33) and seem to be among the first to separate.

In the metaphase the chromosomes which divided first tend to be oriented with their long axes along the spindle, but, due to lack of synchronism in their division, not all of them are so oriented at this stage. Thus they appear more often to form merely a cluster rather than a definite plate. In figure 34, plate 29, the chromosomes are most nearly arranged so as to give the impression of an equatorial plate.

The intradesmose in the metaphase appears as a fine strand connecting the polar masses, in a meridional position inside the nuclear membrane. If not above or below the spindle, it is often distinguished with difficulty from the nuclear membrane.

ANAPHASE

Figure G; plate 29, figures 35, 36

In the late metaphase or early anaphase the sixth chromosome divides, so that there are twelve daughter chromosomes. These separate in two groups of six each, and move to their respective poles. They do not move in definite plates, but usually with one or two chromosomes preceding, and one lagging.

Late anaphases are lacking in the series. In the two available early anaphases (pl. 29, figs. 35, 36) the nucleus appears to be somewhat flattened, and the axis of the spindle extends along neither the greatest nor the shortest diameter of the nucleus, but at an angle between them. In the region of the polar masses, the nuclear membrane curves or bulges outward in a broadly conical fashion. The polar caps are concavo-convex masses against the nuclear membrane. The intradesmose is as in the metaphase.

TELOPHASE

Figures H, I; plate 29, figures 37–39

Early telophases are lacking in the series. In the earliest available telophase (pl. 29, fig. 37) the daughter nuclei have just separated and moved to opposite poles of the amoeba. The nuclear membrane at the point of separation is drawn out as a long tube whose rupture in a central plane perpendicular to its long axis constitutes the actual division of the nucleus. In the specimen figured, the rupture is not yet closed off, so that each daughter nucleus is roundly flask-shaped. Six chromosomes are closely grouped in each daughter nucleus, and in one, these are beginning to lose their individuality. The polar masses are much decreased in size. A large amount of chromatin material is massed on the periphery in the region of the rupture, forming a tube within the attenuated neck of the flask. Strands extend from the chromosomes to this chromatin mass. The appearance of this mass simultaneously with the decrease in size of the large polar cap suggests that the polar cap may contribute in part to its substance.

To derive this stage (pl. 29, fig. 37) from the preceding one (fig. 36) we must assume that as the chromosomes approach the polar masses in the anaphase, the nucleus greatly elongates, and constricts in the equator; that the two halves pull apart before their walls are closed at the point of rupture, so that the nuclear membrane is drawn out in a cylindrical tube across the center of which the rupture occurs; that simultaneously each polar cap decreases in size, and that chromatin derived possibly from its substance becomes concentrated in the region of the rupture. Specimens whose nuclei are in the late anaphase and early telophase are needed to verify these assumptions, and to explain certainly the origin of the chromatin which is concentrated in the region of separation.

The details of the reorganization of the daughter nuclei are not completely known. Material is insufficient, and nuclei in the available late telophases stain very lightly so that they are difficult to study. The amoeba elongates, the nuclei occupying positions at the ends of the long axis. The break in the membrane of each nucleus becomes closed, and the mass of chromatin in its vicinity, as such, disappears. It probably goes to form in part the peripheral chromatin of the new nucleus, for this reappears in small blobs ranged about the inner surface of the nuclear membrane. The chromosomes decrease in size and disappear; fibers suggesting new spoke radii (pl. 29, fig. 38) simultaneously reappear. The origin of the new karyosome is not evident in available specimens.

The cytoplasm of the amoeba constricts in the equatorial plane, appearing granular and less deeply staining in the region of constriction (pl. 29, fig. 39) and division takes place forming two daughter amoebae.

SUMMARY

1. The food of *Endamoeba gingivalis* consists chiefly of "salivary corpuscles," which are the naked nuclei of disintegrating leucocytes. Also, to an extent as yet undetermined, this amoeba ingests whole leucocytes of a polymorphonuclear type. Ingested leucocytes are contained in large vacuoles, in which they are surrounded by a clear fluid. Digestion is rapid. The cytoplasm of the digesting leucocyte loses its structure and becomes a dark-staining, granular mass surrounding the digesting, black-staining lobes of the nucleus. The cytoplasm disappears, and the liquefying lobes of the nucleus fuse in a single spherical mass which decreases in size as it is digested. In late stages of digestion such remains would be indistinguishable from those of ingested "salivary corpuscles." It is possible, therefore, that ingestion of whole leucocytes is more frequent than recognizable specimens would indicate. These findings disprove the conclusions of Goodey and Wellings, who do not find evidences of ingestion of whole leucocytes and who conclude that the amoeba is of no pathogenic importance. The fact that *E. gingivalis* destroys leucocytes suggests a possible pathogenic rôle of the amoeba, and indicates that the subject of its pathogenicity is not a closed one.

2. Several polymorphonuclear leucocytes sometimes unite to form a giant cell enclosing an amoeba in a common vacuole.

3. Viscous, liquid food inclusions of leucocytic origin may be expelled from their containing vacuoles under certain unknown conditions of stimulation. Extrusions occur usually from one vacuole only, but may occur from two vacuoles. Amoebae frequently ingest the free ends of extrusions from other amoebae, so that the two organisms are connected by a strand of siderophile material.

5. In the first mitotic figures available, the karyosome as such has disappeared. Six chromosomes appear as small granules on the spoke radii. These increase in size and migrate toward the center of the nucleus, where they appear as a cluster of minute, blunt rods. They seem to derive their chromatin from the peripheral chromatin and from the granular halo, which simultaneously disappear. The beads of peripheral chromatin remaining after chromosome formation become concentrated in a single peripheral mass, carrying with them the spoke radii, so that these converge into the mass, connecting the chromosomes with it. The chromosomes split longitudinally, but not

synchronously. The peripheral mass or centrosome divides, and the daughter centrosomes migrate to positions diametrically opposed to each other. As they do so they draw out between them a fine strand or intradesmose, which lies in a meridional position inside the nuclear membrane. Stretched between the poles along their axis is the spindle, bearing the clustered chromosomes in an equatorial position. The chromosomes separate and migrate toward their respective poles. One pair of chromosomes is smaller than the others. As the chromosomes approach the poles, the nucleus constricts in the equatorial plane, and divides to form two daughter nuclei. The polar mass in each breaks up and its chromatin becomes redistributed on the inner surface of the nuclear membrane. The chromosomes disappear, probably contributing to the new peripheral chromatin. The spoke radii reappear. The origin of the new karyosome is not known. The amoeba elongates and constricts in the equator, dividing to form two daughter amoebae.

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EXPLANATION OF PLATES

Figures are camera lucida drawings of *Endamoeba gingivalis* from smears of human gingival exudate fixed in Bouin's fluid and stained with iron haematoxylin. × 2500 diameters unless otherwise stated.

PLATE 25

Fig. 1. Amoeba ingesting leucocyte. The amoeba is thrusting out three (visible) small pseudopodia which are surrounding the leucocyte.

Fig. 2. Amoeba ingesting leucocyte. The portion of the leucocyte already ingested is contained in a large vacuole. The nucleus, and the cytoplasm of the ingested portion have undergone partial digestion, with loss of structure and altered stainability. A flare of uningested cytoplasm with typical structure protrudes through an opening in the vacuole and cell wall.

Fig. 3. Amoeba with remains of ingested polymorphonuclear leucocyte. The several black-staining lobes of the leucocytic nucleus are visible, surrounded by a dark-staining, structureless mass of disintegrating cytoplasm.

Fig. 4. Amoeba containing remains of ingested leucocyte. Only a thin film of leucocytic cytoplasm surrounds the nucleus, one lobe of which is breaking up.

Fig. 5. Amoeba with remains of ingested leucocyte. The cytoplasm has entirely disappeared. The several lobes of the polymorphic nucleus remain.

Fig. 6. Amoeba containing remains of ingested leucocyte. The cytoplasm has been entirely digested. The liquefying lobes of the polymorphic nucleus are losing their ovoid shape, and together are forming a spherical mass.

Fig. 7. Amoeba with remains of ingested leucocyte. The spherical mass consists of lobes of the nucleus, which have almost completely lost their individuality.

Fig. 8. Three polymorphonuclear leucocytes actively surrounding an amoeba. Two leucocytes are above, one below the amoeba, which is thrusting out two small pseudopodia.

Fig. 9. Amoeba containing recently ingested nucleus of leucocyte whose cytoplasm had disintegrated previous to ingestion. The structure of the nucleus has not yet been visibly altered.

Fig. 10. Three polymorphonuclear leucocytes have formed a giant cell surrounding an amoeba which is contained in a vacuole common to their bodies. The nucleus and cytoplasm have not been visibly altered, but there is an unusually heavy cell wall.

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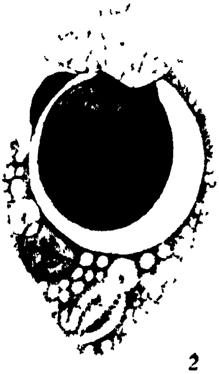

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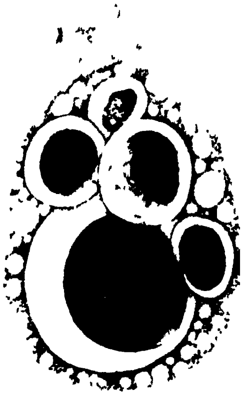

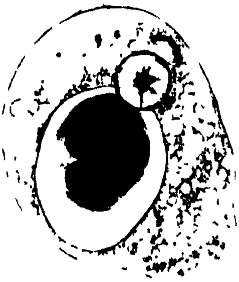

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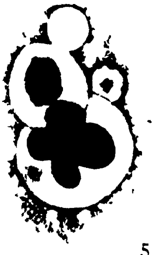

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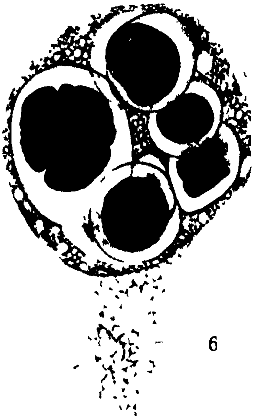

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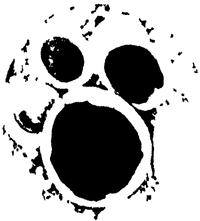

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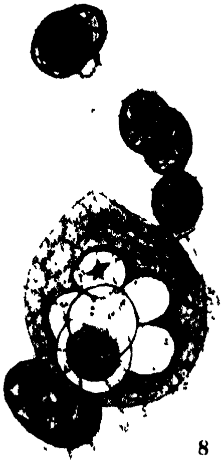

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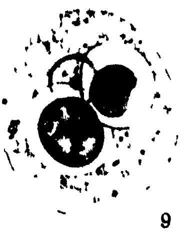

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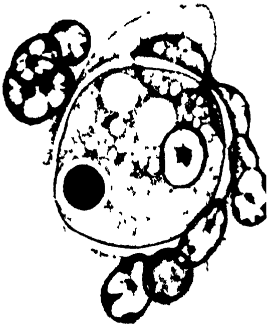

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PLATE 26

Fig. 11. Two amoebae connected by strand of siderophile substance egested by one amoeba and ingested by the other.

Fig. 12. Amoeba with two siderophile extrusions. On each extrusion there is a hump or flare just outside the amoeba.

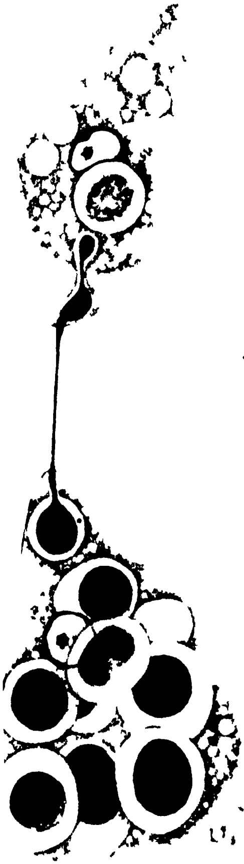
Fig. 13. Amoeba with single siderophile strand, the free end of which has been ingested by a leucocyte. Two lobed food inclusions suggest nuclei of polymorphonuclear leucocytes whose cytoplasm had disintegrated previous to ingestion by the amoeba.

Fig. 14. Amoeba with siderophile substance ejected from single vacuole. There is a hump on the strand within the amoeba, and the strand terminates in a knob.

Fig. 15. Two amoebae connected by siderophile strand. The amoebae are touching each other, so that the smooth strand is entirely within their bodies.

Fig. 16. Two amoebae connected by two siderophile strands. The amoebae are touching, so that only a small portion of one strand is free from their bodies.

Fig. 17. Amoeba, 7 microns by 9 microns. Strand, 56 microns long. Note typical hump just outside the amoeba.



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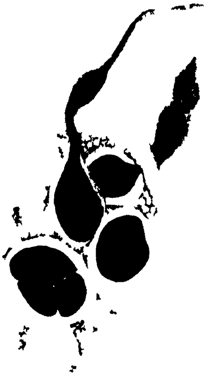

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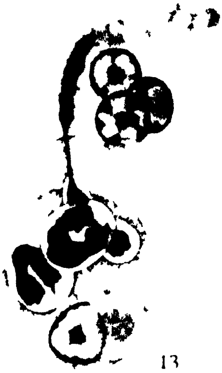

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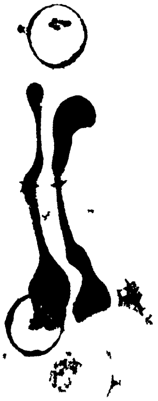

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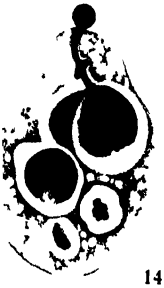

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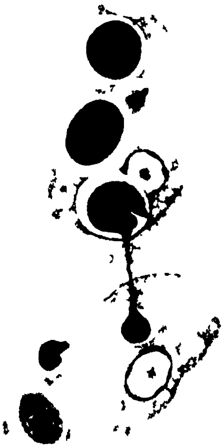

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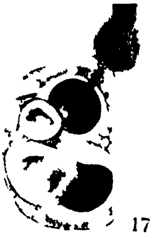

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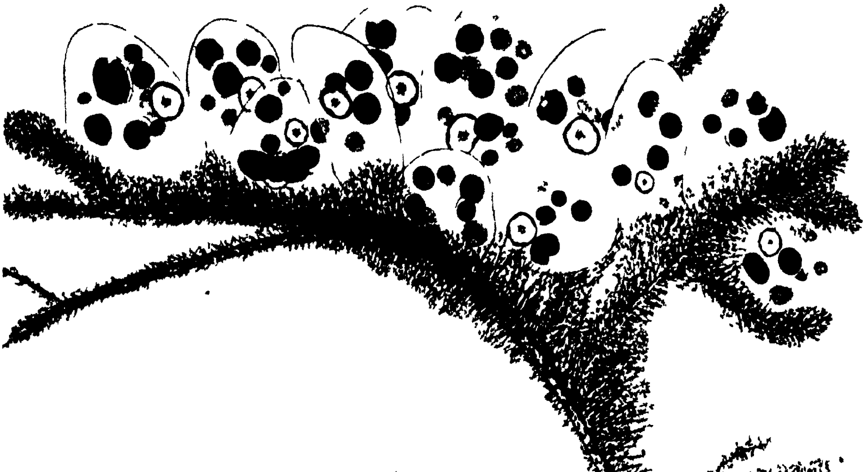
PLATE 27

Fig. 18. Group of amoebae associated with mass of *Leptothrix*. Compare Goodrich and Moseley (1916, pl. 14, fig. 14) and Kofoid and Swezy (1924, pl. 16, fig. 15). × 1360 diameters.

Fig. 19. Amoebae associated with cluster of fusiform bacilli.

Fig. 20. Three amoebae, one with siderophile connections with each of the other two.



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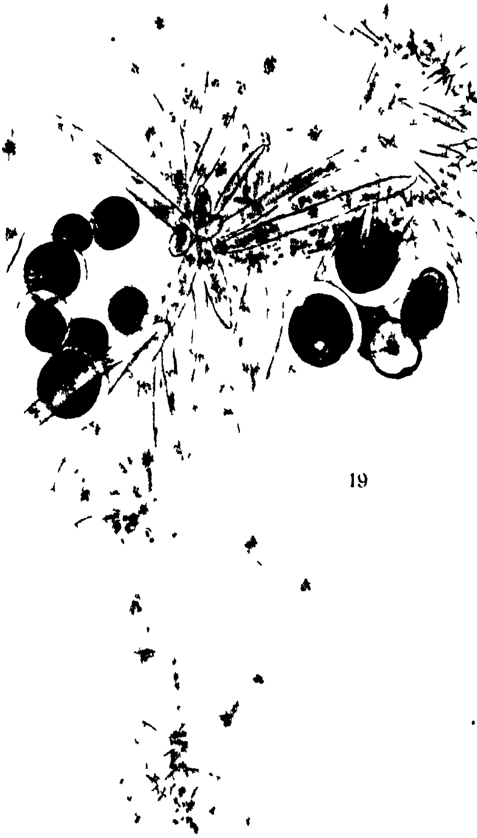

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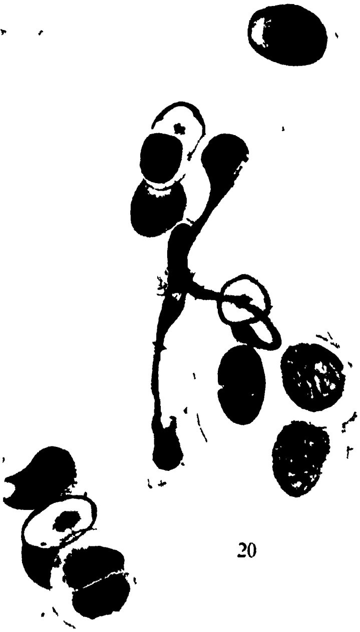

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PLATE 28

Fig. 21. Amoeba with nucleus in the resting stage, showing karyosome and granular halo, spoke radii and peripheral chromatin.

Fig. 22. Early prophase. Six small chromosomes appear on the spoke radii. A vestige of the granular halo remains.

Fig. 23. Prophase. The chromosomes are approaching the center of the nucleus.

Fig. 24. Prophase. The chromosomes have bunched together in a central position. Spoke radii connect them with the beads of peripheral chromatin.

Fig. 25. Prophase. The peripheral chromatin is becoming concentrated on one side of the periphery of the nucleus. Spoke radii connect the chromosomes with the beads of peripheral chromatin. Fragments of the granular halo remain.

Fig. 26. Prophase. The peripheral chromatin has become concentrated in a single mass, from which fibers extend to the six chromosomes.

Fig. 27. Prophase. Two chromosomes have divided and a third is dividing.

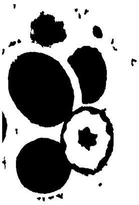
Fig. 28. Prophase. Four chromosomes have divided and a fifth is dividing. The centrosome or peripheral mass of chromatin is notched, so that it appears slightly bilobed.

Fig. 29. Late prophase. The centrosome has divided, and the daughter centrosomes are separating.

Fig. 30. Late prophase. The centrosomes have still further separated. The connecting intradesmose is closely applied to the nuclear membrane.

Fig. 31. Late prophase. Seen from above the position of the original polar mass. The centrosomes or polar masses have migrated to positions not quite diametrically opposed to each other, and are therefore in a plane above the optical section of the sphere at its greatest diameter.

Fig. 32. Metaphase. There is a definite spindle, and eleven chromosomes. One pair of chromosomes is smaller than the others. The intradesmose is closely applied to the nuclear membrane in the upper arc of the optical section represented.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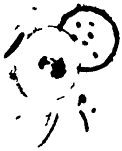
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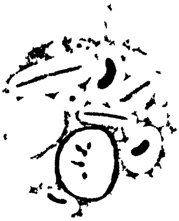
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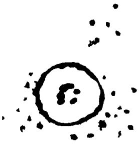
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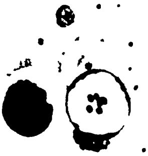
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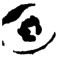
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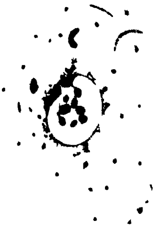
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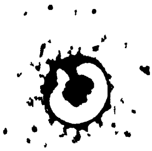
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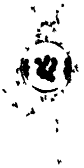
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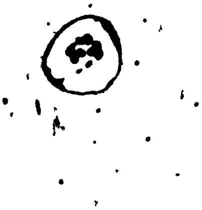
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PLATE 29

Fig. 33. Metaphase. Two chromosomes are definitely smaller than the others. The heavy line connecting the polar caps is the intradesmose.

Fig. 34. Metaphase. The chromosomes form a fairly definite plate.

Fig. 35. Early anaphase. The granule between the nuclear membrane and one polar cap suggests a centrosomal granule other than the polar cap itself. The intradesmose extends from this pole diagonally backwards, in a meridional position under the nuclear membrane above the spindle as oriented in the figure. Note pair of small chromosomes.

Fig. 36. Later anaphase. The polar caps are broadly cup-shaped, rounded with the contour of the nuclear membrae. The amoeba is pinching off(?) an anucleate bud, in which the cytoplasm is in a state of marked degeneration.

Fig. 37. Telophase. The daughter nuclei have just separated, and the breaks in their membranes are not yet closed. Polar caps as such are disappearing. Chromatin substance appears massed in the region of the rupture in each nuclear membrane. Six chromosomes in each daughter nucleus are closely grouped, and are beginning to lose their individuality.

Fig. 38. Later telophase. The nuclei are becoming reorganized. The ruptures in the membranes are closed. The chromosomes are disappearing. Peripheral chromatin is reappearing. Fibers suggest reappearing spoke radii. The cytoplasm is slightly constricted.

Fig. 39. Late telophase. Peripheral chromatin is reappearing. Karyosomes are becoming reorganized. Cytoplasm is well constricted.

Fig. 40. Amoeba 8 microns in diameter. Atypical nucleus. Possibly product of recent division.

Fig. 41. Amoeba 6 microns by 8 microns. Typical nuclear food inclusions of leucocytic origin.

Fig. 42. Free anucleate bud with food inclusion.

Fig. 43. Amoeba pinching off(?) anucleate bud. The nucleated amoeba contains an ingested fusiform bacillus.

Fig. 44. Amoeba containing long fusiform bacillus. The long protruding end of the rod is enclosed in a thin film of cytoplasm.

Fig. 45. Binucleate amoeba 35 microns long by 9 to 20 microns wide. Compare figure 41. Both X 2500 diameters.

Fig. 46. Amoeba with ingested coiled alga-like filament. Amoeba 9 microns by 16.5 microns. Length of ingested portion of filament, approximately 90 microns.

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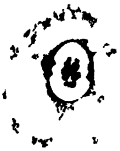
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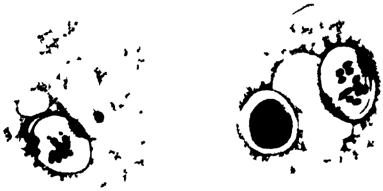
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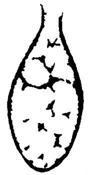
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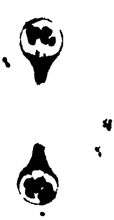
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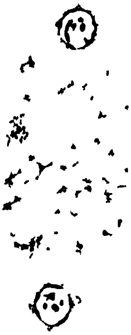
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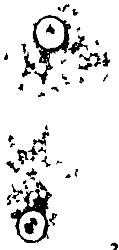
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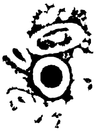
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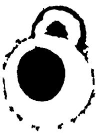
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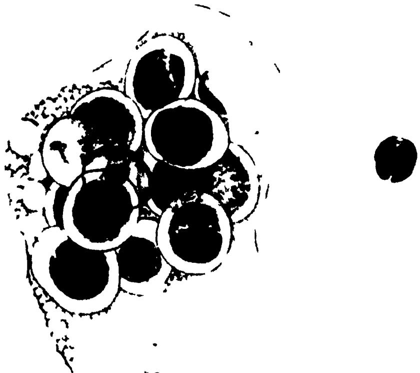
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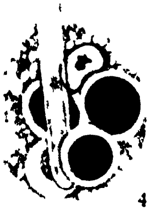
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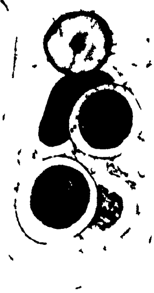
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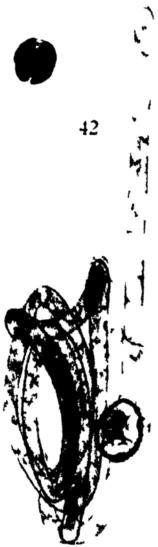
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ON OXYMONAS, A FLAGELLATE WITH AN EXTENSILE AND RETRACTILE PROBOSCIS FROM KALOTERMES FROM BRITISH GUIANA

BY

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CONTENTS

	PAGE
Introduction	285
Oxymonas projector sp. nov.	286
Occurrence	286
General morphology	286
Evolutionary development and relationships	293
Oxymonas..	295
Oxymonas pediculosa sp. nov.	296
Oxymonas gracilis sp. nov.	297
Summary	298

INTRODUCTION

Among the many diverse and elaborate structural adaptations of the Mastigophora to the environment of the digestive tract of xylophagous termites, none is more unique than the development of an anterior proboscis capable of extension and retraction. Its anterior location involves either the lateral dislocation of the normally anterior flagellar complex, or its extinction. In its function the proboscis appears to replace, at least in part, that of the flagella, and, in the evolution of these flagellates in other genera than *Oxymonas*, ultimately, to lead to the entire elimination of flagella, the basic structural features of the Mastigophora.

This paper is an account of several extraordinary flagellates in which the neuromotor system is involved in the structure and function of a retractile and extensile proboscis which functions as an organ for movement and also as an organ of insertion and attachment to the digestive wall of the host. Four species have been found in the intestinal contents of *Kalotermes,* of which three occur in termites collected for us by Professor Alfred Emerson at the Biological Station at Kartabo, British Guiana, in the summer of 1920. Smears of the intestinal contents were fixed in Schaudinn's fluid and later stained by us in iron haematoxylin.

Oxymonas projector sp. nov.

Pl. 30, figs. 1–8; figs. A, B, C

OCCURRENCE

Oxymonas projector occurs in all of the smears which were made from the nymph and soldier castes of *Kalotermes* (*Glyptotermes*) *perparvus* Emer. from Kartabo. The numbers of individuals present on the several slides, each of which contains the intestinal contents of one host, are usually not large. They are frequently found in contiguous regions as though detached in groups in the removal of the intestinal contents. In one instance a group was found in which each individual had its proboscis in contact with or between epithelial cells.

GENERAL MORPHOLOGY

The body of this organism is subject to considerable changes in form depending upon the degree to which the proboscis is protruded or retracted. It is subovoidal to subellipsoidal in contour, pointed posteriorly. In the extended state the anterior end tapers abruptly (pl. 30, fig. 6; fig. C) into a short cone which is continued in a long proboscis of uniform caliber. In the contracted stage (pl. 30, figs. 1, 4; figs. B, C) the anterior end is broadly rounded or forms a low cone, and in some instances (fig. A; pl. 30, figs. 1, 4) the posterior end of the axostyle projects as a slender pointed rod for a distance which may even equal the diameter of the nucleus. In some contracted individuals there may be concavities in the lateral outline in the middle third of the body (figs. A-1, C-4; pl. 30, figs. 1, 3), as though a localized contraction of the cytoplasm had set in. The proportions of the body proper exhibit a wide range indicating considerable mobility (figs. B, C). The changes observed from stout (pl. 30, figs. 1, 3) to slender (fig. 6) phases are not in all cases accompanied by corresponding phases of protrusion or retraction of the proboscis.

The proportions of the body proper range from subspheroidal, stout forms to slender ones of 2.5 transdiameters. In size it may vary

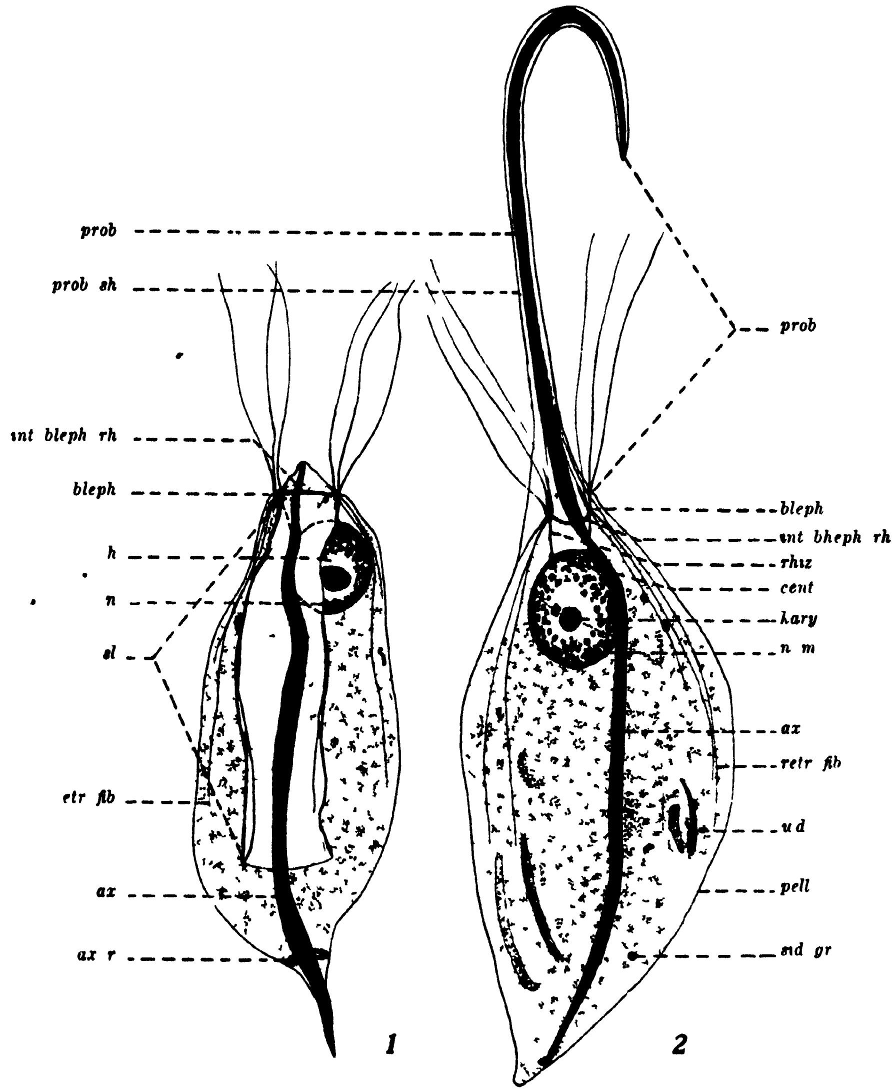

Fig. A. *Oxymonas projector* sp nov. from *Kalotermes perparvus* × 2500. Abbreviations: *ax.*, axostyle; *ax. r.*, axostyle ring; *bleph.*, blepharoplast, *cent.*, centrosome; *h.*, halo around karyosome; *int. bleph. rh.*, interblepharoplast rhizoplast; *kary.*, karyosome; *n.*, nucleus; *n. m.*, nuclear membrane; *pell.*, pellicle; *prob.*, proboscis; *prob. sh.*, proboscis sheath; *retr. fib.*, retractor fibers; *rhiz.*, rhizoplast; *sid. gr.*, siderophile granules; *sl.*, sleeve of proboscis; *wd.*, wood particles in food vacuoles.

1. Contracted individual with proboscis retracted and sleeve condensed and visible.

2. Proboscis extended and sleeve not visible.

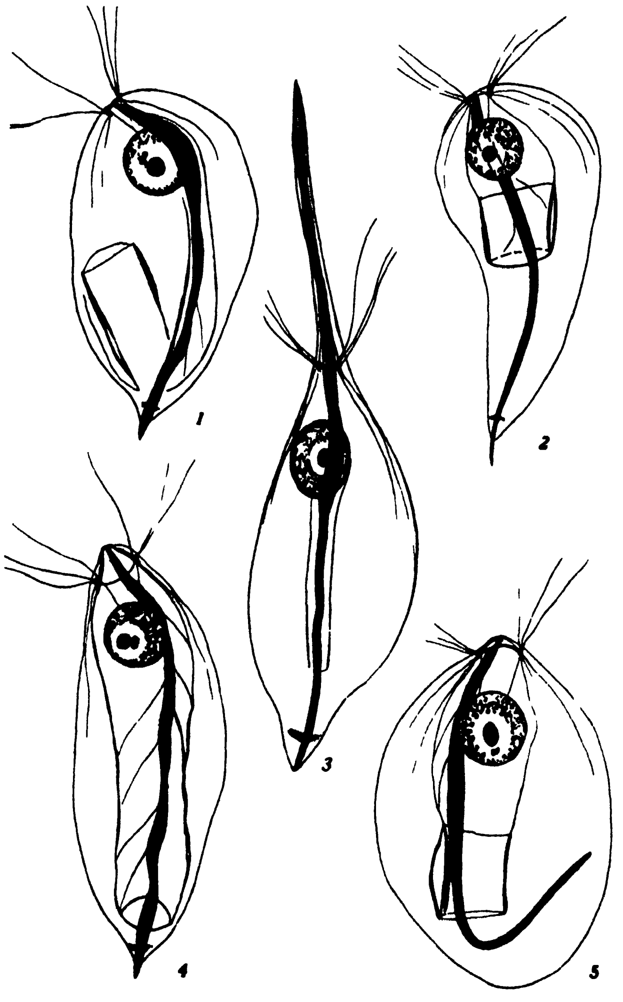

Fig. B. *Oxymonas projector* sp. nov. from *Kalotermes perparius* showing different phases of proboscis and sleeve. × 1800.

1. Proboscis retracted, traces of oblique retractor fibrils from sleeve to axostyle; inverted extra sleeve loose in cytoplasm probably of ancestral origin and discarded after antecedent binary fission.

2. Proboscis retracted, sleeve appearing to be reflexed from posterior end anteriorly.

3. Proboscis partially extended, sleeve faintly visible enclosing a clear column around the axostyle.

4. Proboscis retracted, large expanded sleeve extending nearly the whole length of the animal. Oblique retractor fibrils visible within the sleeve.

5. Much contracted individual, sleeve visible, dilated anteriorly, reflexed (?) posteriorly; axostyle withdrawn within the cytosome, no axostylar ring.

from 12 to 40 microns in length. The proboscis when fully extended is equal to or may even exceed the body proper in length by as much as 3.3 times, measured from the posterior tip to the blepharoplast.

The *cytosome* presents no peculiar contents such as the granules of *O. granulosa* Janicki. The material within the sleeve is less definitely alveolar and as a rule does not contain any particles of wood. Outside of the sleeve it is uniformly and rather finely alveolar and contains small fragments of wood enclosed in small fluid-filled vacuoles (*wd.*, figs. A, B; pl. 30, fig. 6). No evidence of bacterial feeding has been seen. In a few cases (pl. 30, fig. 4) a few very small, siderophile subspheroidal granules of metabolic origin are scattered through the cytoplasm (*sid. gr.*, fig. A). A thin pellicle (*pell.*, figs. A, B) covers the periphery.

The *nucleus* (*n.*, figs. A, B) is almost constantly located near the anterior end about half its own diameter posterior to the blepharoplasts a little to one side of the axis. Its structure is strikingly similar to that of *Oxymonas granulosa* as described by Janicki (1915). It is spherical with a distinct membrane (*n. m.*, figs. A-1, 2), a peripheral zone of coarsely and rather uniformly granular chromatin, a spheroidal or subspheroidal karyosome (*kary.*), the "*Binnenkörper*" of Janicki, in a clear halo (*h.*). This halo is often posteriorly off center and its centrally located karyosome is spherical or subspherical and in some nuclei sends out a few faint spoke radii into the zone of chromatin granules. In one case of postmitotic nuclei the center of the karyosome had a clear area with a granule. At one side of the karyosome lying free in the clear halo is a spheroidal or bilobed body similar to that figured by Janicki in *Oxymonas granulosa* and called by him an "Entosom" which forms a sort of a centrodesmose at mitosis.

The *neuromotor system* of *Oxymonas projector* consists of two sets of three anterolateral flagella, two lateral blepharoplasts (*bleph.*, fig. A-1, 2) connected by a semicircular, horizontal, interblepharoplast rhizoplast (*int. bl. rh.*) from which rhizoplasts pass to two centrosomes (*cent.*) on the anterior nuclear membrane. In addition there is an axial extensile axostyle (*ax.*) and a fibrillar retractor apparatus or sleeve (*sl.*). There is no cytostome.

This genus exhibits, in the organization of its neuromotor system, the thus far unique feature of a duplicated set of anterior *flagella* of the *Monocercomonas* type, namely, each set is three in number. There is, however, no duplication of the axostyle. These anterior flagella arise in two opposite lateral groups near the anterior end at the level where the extension of the proboscis begins. The three flagella are equal and similar in structure and are somewhat shorter than the body in length. Each group appears to arise from a single horizontally elongated and curved blepharoplast. It is possible in some views to see that the two ends of each blepharoplast give rise respectively to one and two flagella homologous with the principal and accessory anterior flagella of *Trichomonas* and *Chilomastix*, but a subdivision into two granules is not evident. The two blepharoplasts lie at the ends of a fibrillar semicircle near the anterior end, below the proboscis. This semicircle, composed of the blepharoplasts and their connecting rhizoplast, appears to be fixed in location and to be distinctly heavier than the rhizoplasts running to the centrosomes. From each of the two blepharoplasts a slender fibril, the rhizoplast (*rhiz.*, fig. A-1, 2) passes posteriorly to a centrosome (*cent.*) on the nuclear membrane. A minute spherical body on or imbedded in the nuclear membrane is found with difficulty.

The *axostyle* is a siderophile, fibrillar, contractile strand extending throughout the main axis of the animal from the anterior tip to the posterior one beyond which it sometimes (pl. 30, figs. 1, 4) seems to protrude or to push the pellicle. The posterior cone in which the axostyle protrudes sometimes contains an axostylar siderophile ring (*ax. r.*, fig. A-1, 2) in the cytoplasm which encircles this organelle near its termination. Axostyles which distend the pellicle posteriorly sometimes retain this ring near the tip.

The fibrillar connections or axostylar rhizoplasts joining the axostyle to the blepharoplast consist of a few fibers passing from the blepharoplasts to the axostyle out into the extended proboscis. There is abundant evidence that the axostyle can increase and decrease its length (fig. C). Its elongation accompanies the extension of the proboscis and its shortening the retraction. In the contracted state its diameter is somewhat greater than in extension. When its course is followed by careful focussing in lateral view or in end view it is seen to pursue a steep spiral course, though not of uniform slope throughout. It terminates anteriorly in direct contact with the pellicle. It appears to be composed of fibrillar strands which exhibit varying degrees of dispersal and concentration in the proboscis under different conditions of extension.

PROBOSCIS

The proboscis (*prob.*) and fibrillar sleeve (*f. sl.*, figs. A, B, C; pl. 30, figs. 1–10) present a great variety of appearances, positions, and degrees of definiteness of structure which appear to be correlated with the movements of extension and retraction of the proboscis. The sleeve (*sl.*, fig. A) is most evident in the retracted state of the proboscis in which it forms a cylindrical structure surrounding the axostyle with clear or nearly clear contents, definite and abrupt posterior end, and much less definite anterior limits. Posteriorly the wall is composed of siderophile substance, which in optical cross-section (pl. 30, fig. 5) seems to form a continuous sheet which locally and especially posteriorly appears to be doubled. In some instances it may even appear to be doubled back upon itself from the posterior end (fig. B-2), but this appearance may be due to other causes. We find no doubling and far less evidence of continuity and distinctness, anteriorly. In a few instances only have the contents of the sleeve stained as deeply as the cytosome generally, and rarely has cytosomal alveolar structure been seen within even the end of the sleeve. The sleeve is traversed in the contracted state by fibrils running from its inner face obliquely anteriorly to join the axostyle (fig. B-4; pl. 30, figs. 4, 5). Their location, and position in retraction, indicate that they are retractor fibrils.

In the extension of the proboscis the axostyle elongates, the clear area within the sleeve decreases in diameter (pl. 30, fig. 3), and the siderophile wall of the sleeve decreases in thickness and distinctness until in the fully extended state (fig. C-6) no trace of the sleeve is in evidence beyond the faint hint of the clear zone sometimes found around the axostyle. It is probable that the fibrillar sheath is carried out with the proboscis and stretches so thin as not to be visible.

In addition to this structure there is also a radiating fan of retractor fibrils (*retr. fib.*) spreading from the axostyle at the level of the blepharoplast semicircle under the pellicle, which forms an essential part of the retractor apparatus.

The interaction of these parts in the movement of the proboscis appears to be as follows. The apical region anterior to the semicircular blepharoplast ring must be extraordinarily elastic, yielding upon pressure exerted by either the contraction of the body toward the sphere, or to the elongation of the axostyle, or both, until full extension (fig. C-6) is accomplished. With this process the fluid of the clear

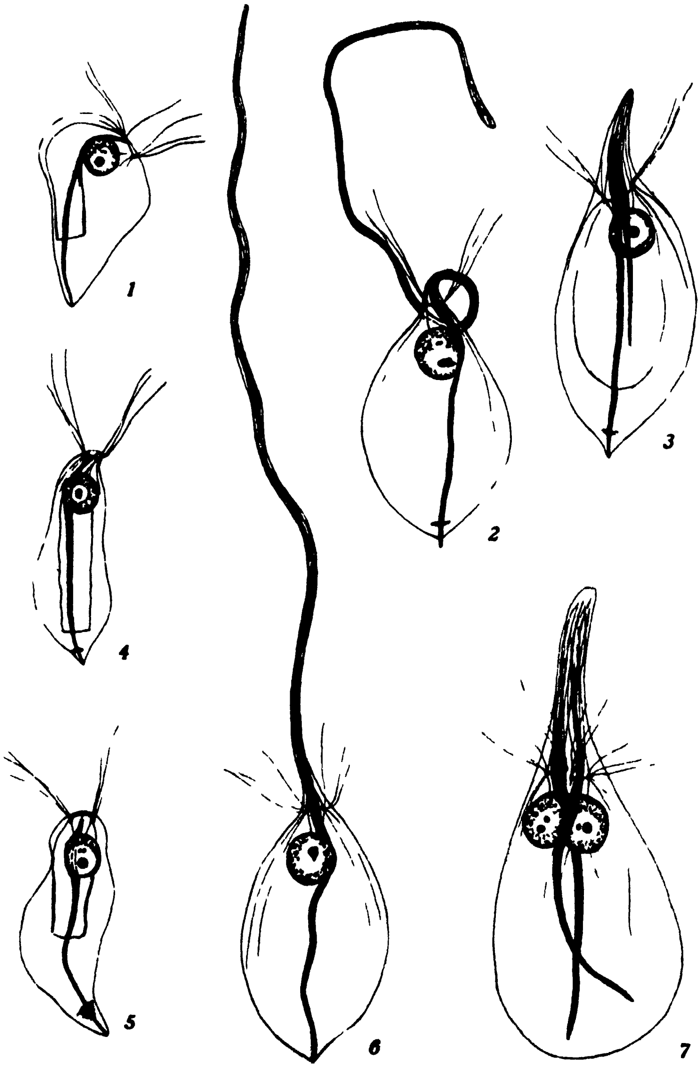

Fig. C. *Oxymonas projector* sp. nov. from *Kalotermes perparvus* Emer., showing changes in cytosome and protrusion of proboscis. × 1000.

1. Cytosome bulging anteriorly, proboscis fully retracted, sleeve evident.

2. Cytosome contracted into a biconical form, proboscis fully extended and somewhat coiled. No sleeve visible.

3. Proboscis slightly extended. Sleeve distended.

4. Proboscis fully retracted. Sleeve at maximum length and definiteness.

5. Proboscis fully retracted. Sleeve very distinct.

6. Cytosome biconical, proboscis at maximum extension, no sleeve visible.

7. Binary fission in progress. Nuclear division completed. Two axostyles in *one* proboscis. Plasmotomy not yet under way.

area within the sleeve is carried out in the extending proboscis and the sleeve vanishes by extension into the same structure. The flagella, blepharoplast semicircle, and nucleus remain undisturbed in these profound shiftings of the other structures. Throughout all the varied aspects of this extraordinary process there are evidences of the spiral course of the axostyle and the great mobility of the cytosome. It is evident that this structure is a powerful organ for pushing through the milieu of flagellates which fill the digestive tract of the host and even of forcing the proboscis into or between the cells of the digestive tract.

There is some evidence that the fibrillar sleeve is a structure of some consistency and persistence. In figure B-1 there lies in an inverted position an extra sleeve in addition to the one around the axostyle. This is doubtless the one discarded at mitosis and belongs to the preceding generation.

EVOLUTIONARY DEVELOPMENT AND RELATIONSHIPS

This genus is fundamentally of the triflagellate type. It is thus one of the simpler polymastigote flagellates with three equal undifferentiated anterior flagella grouped characteristically as a single flagellum and a pair, and a single axostyle. It has diverged from this simple state seen in *Monocercomonas* in two directions, neither of which involves any structural change in the flagella. The first modification is the duplication of the neuromotor system from the centrosome through the rhizoplast-blepharoplast-flagella complex. There has been, however, no accompanying duplication of the axostyle or, by mitosis, of the nucleus. A related process has taken place in the genus *Stephanonympha,* a multicellular flagellate of the termites to which each nucleus is attended by its attached neuromotor complex but the whole anterior end of the animal is covered by a series of detached neuromotor units which continue the orderly arrangement of the nucleus-neuromotor units. In *Oxymonas projector,* in which the neuromotor system loses its *unitary* interdependence with the nucleus and assumes a binary one, a sort of permanent promitotic status, a first step is taken in the detachment of or independence of the neuromotor complex from its unitary association with one nucleus, as in most flagellates. The culmination of this tendency initiated in *projector* is seen in the multicellular condition and the accessory or morphologically superfluous neuromotor units of *Stephanonympha.*

The second direction of advance is in the development of an anterior process of great length and its protrusion and retraction by the aid of cytosomal contraction and of retractile fibers. It is noteworthy that the only additional development in the neuromotor system in this aspect is the blepharoplast semicircle and the elongation of the axostyle. It is thus the axostyle and its derivatives which have differentiated in structure and function in a most striking fashion.

We have no adequate material in which to follow the history of mitosis as Janicki (1915) has done with *O. granulosa,* a few cases of synyezysis and a binucleate telophase (pl. 30, fig. 9) being the only stages available.

In 1915 Janicki described the genus *Oxymonas* from a termite designated by him as *Calotermes castaneus* Burm., collected by Professor A. Silvestri at Honolulu, H. I. This termite is found normally in the southeastern United States. As described by Janicki (1915), *Oxymonas* is devoid of all flagella, and his account has no reference to, and his figures, with the possible exception of his figure 49, plate 17, give no indications that he saw, the blepharoplasts. However, the proboscis, the nucleus with its karyosome, halo, and lateral granule were so strikingly similar to these structures in our species that we were led to suspect that Janicki had overlooked the flagella and that our species was an *Oxymonas.*

Through the kindness of Mr. C. S. Judd of the Board of Commissioners of Agriculture and Forestry of Hawaii we obtained preserved specimens of the Hawaiian termites including *Neotermes connexus* Snyder, which resembles *Calotermes castaneus* which is not known from Hawaii at present. Upon examining this species we find the flagellates described by Janicki (1915) including *O. granulosa.* Upon examination of smears and sections we find in this species, as we expected, the two groups of flagella, the blepharoplasts and semicircular interblepharoplast rhizoplast, and a faint fibrillar sleeve. It has also the peculiar, distributed granules upon whose presence Janicki based his specific name. The two species, *projector* and *granulosa,* are very similar in proboscis, axostyle, retractor apparatus, neuromotor system, and details of nuclear structure. They clearly belong to the same genus. The retractor apparatus is more highly differentiated in *O. projector* than in *O. granulosa,* and the cytoplasm of the latter is filled with spherical granules which are lacking in all our examples of *O. projector.* It therefore becomes necessary to emend the generic description so as to incorporate these essential characters.

Oxymonas Janicki 1915, emend. Kofoid and Swezy

Polymastigote flagellates with neuromotor system consisting of two centrosomes on the nuclear membrane connected by independent rhizoplasts with two lateral blepharoplasts from each of which three flagella arise, connected by a transverse rhizoplast in the form of a semicircle; nucleus with excentric karyosome in clear halo with a lateral granule in the halo; central axostyle continued anteriorly to apex and capable of being extended in an anterior extensile and retractile proboscis. Type species, *O. granulosa* Janicki 1915 from *Neotermes connexus* Snyder from Honolulu, H. I.

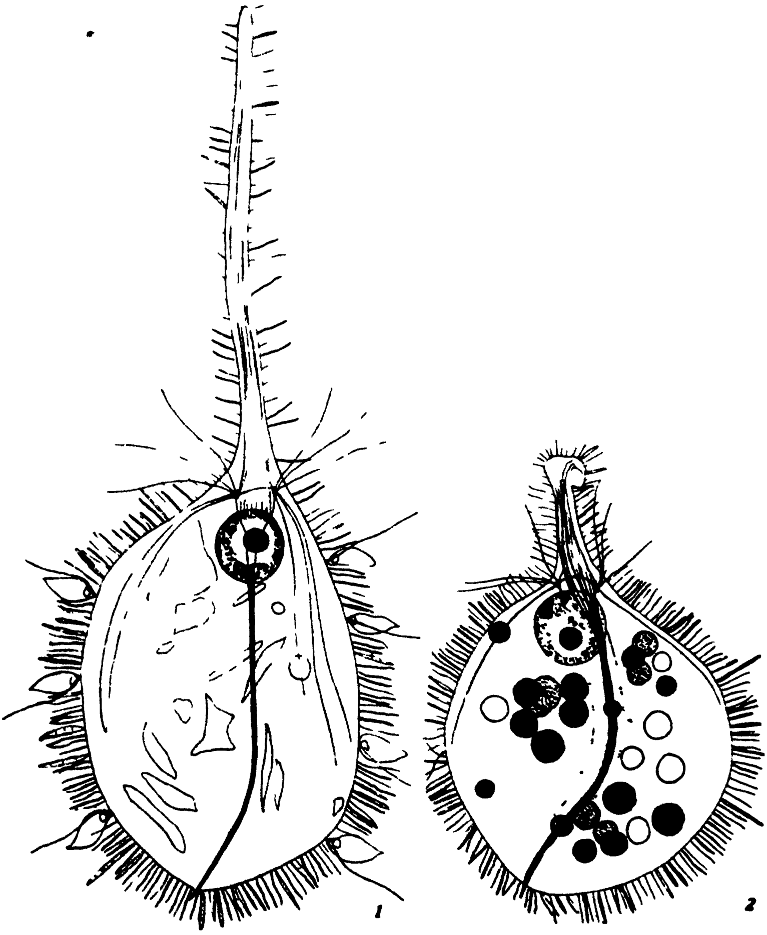

Fig. D. ***Oxymonas pediculosa*** **sp. nov. from** ***Kalotermes nigriceps*** **Emer. × 1800.**

1. Proboscis extended. Body covered with adherent bacteria and small flagellates. No siderophile granules in cytoplasm. No sleeve.

2. Proboscis partially retracted. Sleeve faintly outlined. Cytoplasm with many spherical densely siderophile granules.

Oxymonas pediculosa sp. nov.

Pl. 30, fig. 11; fig. D-1, 2

A third species of this interesting genus has been found by us in intestinal smears from *Kalotermes* (*Lobitermes*) *nigriceps* Emer. from Kartabo made for us by Professor Emerson. The species is very rare, only a few individuals being found in the entire material from any single host. Moreover, each and every one of these flagellates is completely covered by a dense coating of adherent bacteria and often also small flagellates, so that the morphology is usually so obscured as to be made out with difficulty. In many of the individuals the cytosome contains spherical, siderophile granules (fig. D-2) of large size whose dense staining is so marked as at once to differentiate this flagellate from all others in the smears. Not all (fig. D-1) individuals, however, have these granules.

Oxymonas pediculosa has a larger more broadly ovoidal body than *O. projector.* Eight individuals ranged from 27 to 40 (average 34) microns long and 15 to 25 (average 21) wide. The proboscis when extended adds 15 to 39 microns to the length. This species has all the organelles characteristic of the genus. The nucleus is more anterior, the nuclear chromatin is less coarsely granular than in *O. projector*. The lateral granule is very small, the blepharoplasts are smaller, the flagella very difficult to locate on account of the adherent bacteria, the rhizoplasts shorter, the blepharoplast semicircle very faint, the axostyle much more slender, the proboscis relatively shorter and stouter, and the axostyle within it more dispersed. The axostylar sleeve is at the most (pl. 30, fig. 11) faintly outlined as a clear space around the axostyle, terminating abruptly posteriorly as in *O. projector*, but without the deeply stained sheath. The peripheral retractor fibrils spreading posteriorly from the axostyle at the level of the blepharoplasts are present. Particles of wood (fig. D-1) are sometimes abundant, in which case the black spherules are few or absent. The black spherules (pl. 30, fig. 1) are metabolic products which may arise from the wood. Each is enclosed in a vacuole with little intervening fluid, and some of them show evidences of disintegration. They are fewer and relatively larger than those of *O. granulosa* and more siderophile than in any of the other species. The proboscis of this species is relatively stouter, shorter, and blunter than that of the other species, less often curved in fantastic fashion and the axostyle flares suddenly at the level of the nucleus into the fibrillar bundle which stains lightly and fills the stout proboscis. Posteriorly the axostyle projects slightly as a sharp point. The axostylar ring was noted.

Oxymonas gracilis sp. nov.

Pl. 30, figs. 9, 10; fig. E 1–3

In three slides of intestinal smears from the soldier castes of *Kalotermes* (*Rugitermes*) *magninotus* Emer. from Kartabo, prepared for us by Professor Emerson, there occur very small flagellates which clearly belong to another species of the genus *Oxymonas.*

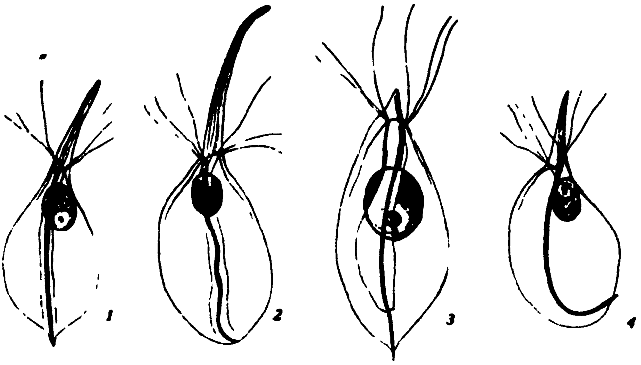

Fig. E. *Oxymonas gracilis* sp. nov. from *Kalotermes magninotus* Emer. × 1800.

1. Proboscis partially extended. Sleeve faintly outlined.
2. Proboscis more fully extended. Sleeve visible as a slender clear zone about the axostyle.
3. Proboscis retracted. Sleeve distended.
4. Proboscis partially extended.

The body has an ovoidal or ellipsoidal shape, pointed anteriorly with a marked tendency to expansion posteriorly (pl. 30, fig. 9). The cytosome is very often distended with fluid-filled vacuoles of large size which crowd the cytoplasm anteriorly (pl. 30, fig. 9). A few individuals have smaller uniform vacuoles with spheroidal bodies which stain lightly (pl. 30, fig. 10). The nucleus is anterior, spheroidal (fig. E-3), or more often ovoidal and compressed anteriorly (pl. 30, fig. 9) in the conical anterior end of the body. The chromatin is more irregularly dispersed and more ragged than in the other species and the halo less distinct. No lateral granules could be with certainty distinguished from the other siderophile structures. The two blepharoplasts are small, far anterior, their interconnecting semicircular rhizoplast is a slender thread, and their nuclear rhizoplasts long, converging on the nucleus.

The proboscis when fully extended attains only the 1.6 length of the body (in our smears) and is either of uniform caliber (pl. 30, fig. 9) or tapers to a more or less pointed tip (pl. 30, fig. 10; fig. E). The axostyle is very slender flaring out in fibrils in the proboscis, often more lateral than axial in location, has a posterior siderophile ring near the pellicle, and may be extended posteriorly (pl. 30, fig. 10).

The sleeve is feebly developed as a slender faintly delineated clear zone around the axostyle when the proboscis is extruded and as a wider but still faintly margined cylinder with definite posterior limit when the proboscis is withdrawn (fig. E-2, 3). In some individuals the partially extruded proboscis may have the form of a broad obtuse stump. The usual peripheral retractor fibrils are present. This is the smallest species of the genus, many individuals being only 8 by 4 microns. The largest we have measured is 25 by 15 microns. The average length and width of seven individuals are 15 and 8.5 microns respectively, but in some hosts the average length is only 12 microns. The longest proboscis, seen on a large individual, was 36 microns long. This species was relatively more abundant in its host than any of the other species were in their hosts.

SUMMARY

Oxymonas projector from the digestive tract of *Kalotermes* (*Glyptotermes*) *perparvum* Emer., from Kartabo, British Guiana, is a uninucleate polymastigote flagellate derived from the triflagellate ancestor by the duplication of the neuromotor system (except the axostyle) without nuclear duplication. Each unit of the duplicated system consists of centrosome, rhizoplast, blepharoplast (united with its mate by a blepharoplast semicircle), and three equal flagella. There is a single axial rhizoplast which extends anteriorly into the extensile and retractile proboscis operated by cytosomal protraction and retractor fibrils attached to the axostyle.

In the genus *Oxymonas* as originally described by Janicki (1915) the flagella were overlooked entirely and the proboscis sleeve was not found. The description of the genus is accordingly emended here to include these essential features.

The nucleus has a posteriorly excentric karyosome with surrounding clear halo in one side of which an endosome is found.

This flagellate presents the first step in the progressive independence of the neuromotor complex from the nucleus. It passes in this genus from a unitary to a partial binary status. A later stage in this process is seen in *Stephanonympha,* in which the somatella is multinucleate but there is a great excess of neuromotor units in the spiral series.

Two other new species, *O. pediculosa* from *Kalotermes nigriceps* Emer. and *O. gracilis* from *K. magninotus* Emer., have similar structures but the proboscis and sleeve are less highly evolved.

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EXPLANATION OF PLATE 30

All figures of *Oxymonas* from *Kalotermes* from British Guiana, from intestinal smears fixed in Schaudinn's fluid and stained in iron haematoxylin.

Oxymonas projector sp. nov. from *Kalotermes perparvus* Emer.

Fig. 1. With proboscis retracted within sleeve, which seems to be telescoped at its posterior end. × 1890.

Fig. 2. Anterior end only with proboscis slightly extended. Some retractor fibers within the sleeve. × 2100.

Fig. 3. Proboscis partially extended. Sleeve scarcely visible. × 1890.

Fig. 4. Proboscis wholly retracted. Retractor fibers both within and without the sleeve clearly visible. Few small siderophile granules in the cytoplasm. × 1890.

Fig. 5. Optical cross-section of body showing retractor fibers in sleeve. × 2100.

Fig. 6. Proboscis much extended. Sleeve vanished. Wood chips in cytoplasm. × 1890.

Fig. 7. Nuclear region only, showing centrosomes on nuclear membrane, retractor fibrils, and nuclear rhizoplasts. × 2100.

Fig. 8. Nuclear division completed, two neuromotor systems, including axostyles and sleeves. Endosomes in nuclear halo. × 1890.

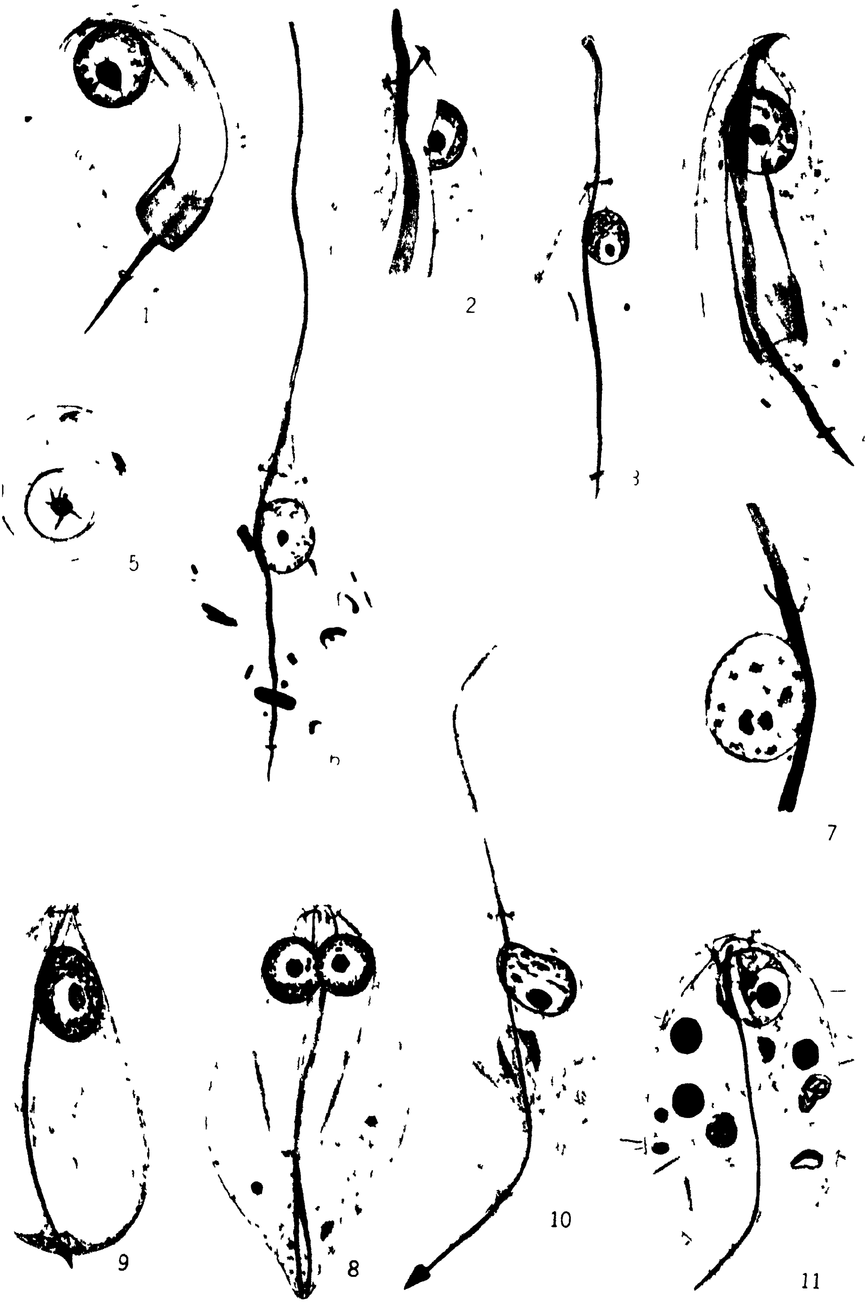
Oxymonas gracilis sp. nov. from *Kalotermes gracilis* Emer.

Fig. 9. Proboscis partially extended. Cytoplasm with large fluid-filled vacuoles. Sleeve almost vanished. × 2500.

Fig. 10. Proboscis more extended. Axostyle projecting posteriorly carrying axostylar ring with it. × 2500.

Oxymonas pediculosa sp. nov., from *Kalotermes magninotus* Emer.

Fig. 11. Proboscis fully retracted. Sleeve outlined as a clear cylinder around the axostyle. Large siderophile granules in cytoplasm. Surface covered with adherent bacteria. × 1890.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ON PROBOSCIDIELLA MULTINUCLEATA GEN. NOV., SP. NOV., FROM PLANOCRYPTOTERMES NOCENS FROM THE PHILIPPINE ISLANDS, A MULTINUCLEATE FLAGELLATE WITH A REMARKABLE ORGAN OF ATTACHMENT

BY

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CONTENTS

	PAGE
Introduction	301
Proboscidiella multinucleata gen. nov., sp. nov	302
Morphology	302
Binary fission	308
Encystment	309
Organization and integration	310
Bionomics	310
Relationships and evolutionary origin	311
Generic characters	312
Summary	312

INTRODUCTION

One of the evolutionary features exhibited in several genera of the flagellates found in the digestive tract of the xylophagous termites is the formation of a multicellular somatella as a permanent or at least a persistent phase. This is seen clearly in such genera as *Stephanonympha* and *Calonympha*. In the former, the nuclei, reduplicated each with its accompanying neuromotor system, including the axostyle and parabasal body, form in a spiral sequence in the anterior end of the body. In the latter, a similar arrangement is found of these same reduplicated structures with the addition of the reduplication of the neuromotor complex without accompanying nuclei in the most anterior part of the spiral. In these genera the cells are numerous, one hundred or more in the former and as many as fifty in the latter.

In this paper we describe a new genus in which the multinucleate stage is attained but the number of nuclei is not so great, inconstant, and quite variable. The proboscis is highly developed in this genus as an organ of attachment, combining in a single structure the anterior extension of the axostyles of all the individual neuromotor systems.

Our material consists of intestinal smears made by Professor S. F. Light, and serial sections of the digestive tract of larvae and nymphs. Both smears and sections were fixed in hot Schaudinn's fluid and stained by us in iron haematoxylin. The host is *Planocryptotermes nocens* Light (1921) from Manila, P. I., where it occurs as a house termite in dry and seasoned timber of houses and of furniture.

Proboscidiella multinucleata gen. nov. sp. nov.

Pls. 31 and 32; figs. A–D

MORPHOLOGY

The *body* is ellipsoidal or ovoidal, and extraordinarily variable in proportions and shape because of its great contractility and protean transformations (fig. B). It may be wider anteriorly (fig. B-1) or posteriorly (fig. B-3), constricted to a dumb-bell shape (fig. B-4) or spread out horizontally in an amoeboid embrace of a wood splinter (fig. D). It varies greatly in size; from a small, subspheroidal form 25 microns in diameter to an elongated, ellipsoidal, contracted form with the body proper 160 microns long and 113 microns in diameter. The average length of 16 individuals was 72 microns. When fully extended the proboscis may have a length two or three times that of the body. An individual 64 by 26 microns had an extended proboscis 160 microns in length.

The *cytoplasm* is coarsely and uniformly alveolar and is often full of particles of wood (pl. 31, fig. 7) in vacuoles, or of numerous spheroidal globules, likewise in vacuoles somewhat evenly distributed throughout the body (pl. 31, fig. 3), or of deeply staining siderophile droplets or other variously shaped masses (pl. 31, figs. 1, 3, 5, 7), or of angular and cylindrical non-staining bodies of unknown origin (pl. 31, figs. 1, 7), probably wood.

In addition to these food particles and products of metabolism, there are also present in a few individuals certain tubular or funnel-shaped structures (pl. 31, figs. 1, 5) which challenge one's imagination for an explanation of their origin and significance. We have been able to find no organisms or food substance in the digestive tract of the host

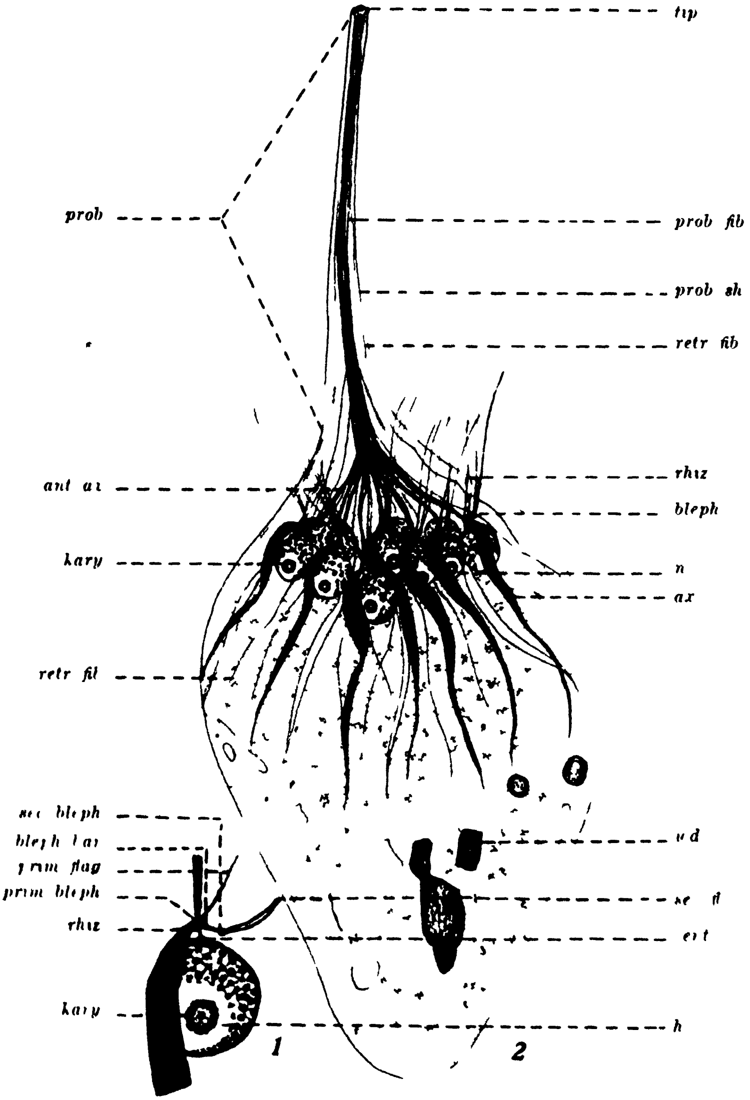

Fig. A. 1, *Proboscidiella multinucleata* gen. nov., sp. nov. Nucleus and neuromotor system. ×2300. 2, Lateral view of an eleven-celled somatella. × 1100. Abbreviations: *ant ax*, anterior extension of the axostyle, *ax*, axostyle, *bleph*, blepharoplast, *bleph bar*, blepharoplast bar or rhizoplast connecting the primary and secondary blepharoplasts, *cent*, centrosome, *chr ret*, chromatin reticulum, *h*, halo, *kary*, karyosome, *prim bleph*, primary blepharoplast, *n*, nucleus, *prim fl*, primary flagellum, *prob*, proboscis, *prob fib*, proboscis fibers, *prob sh*, proboscis sheath, *retr fib*, retractor fibers, *rhiz*, rhizoplast, *sec bleph*, secondary blepharoplast, *sec fl*, secondary flagella, *tip*, end of the proboscis, *wd*, particle of wood.

which might account for these characteristic structures, whose structure suggests an organic origin, while their stainability indicates that they are living substance or its immediate product. They are never enclosed in a vacuole. They are tubular when short, concave funnel-shaped when elongated, with truncate ends and more or less concave sides. They vary in length, diameter, and flare as though they were structures subject to growth or a formative process. They are not localized or in a structural association with any organ of the body.

Some light may be thrown on these problematical structures by the occurrence in *Oxymonas projector* (see Kofoid and Swezy, 1925, p. 288, fig. B 1) of an extra proboscis sleeve, in addition to the one in place around the axostyle. The existence of this extra sleeve indicates a certain independence and persistence, assuming, as we did, that it is a survival from the previous ancestor, which has been functionally replaced by a new sleeve formed at the preceding binary fission. However, we find no sleeves around the axostyles in *Proboscidiella* and these problematical structures do not seem to be the handed-down furniture of the previous generations. They may, however, be vestigial structures from an *Oxymonas*-like ancestor, still formed at times but no longer serving their primal function.

The *nucleus* (fig. A-2, *n.*) is very characteristic. It has a distinct membrane, an elaborate chromatin reticulum (*chr. ret.*), a spherical halo (*h.*) about 0.25–0.40 of the diameter of the nucleus, located posteriorly and containing a siderophile granule, and a central karyosome (*kary.*), 0.35 to 0.50 of the diameter of the halo. The nuclei are always located in the anterior end at the base of the proboscis within a short distance of the pellicle and circlet of flagella.

The number of nuclei is extraordinarily variable and is correlated with the size of the body, though it seems to bear no relation to its form and shape, the larger numbers occurring in the larger individuals. We have found the numbers to range from 1 to 34. The even numbers of nuclei, particularly 4, 6, 8, and 12, are more frequent than the odd numbers. In 50 individuals the numbers of the nuclei were distributed as follows:

Number of nuclei	Number of individuals	Number of nuclei	Number of individuals	Number of nuclei	Number of individuals
1	1	8	6	14	1
2	1	9	3	17	1
3	5	10	3	18	1
4	4	11	3	19	1
5	1	12	5	27	1
6	9	13	1	34	1
7	2	....	....	....	....

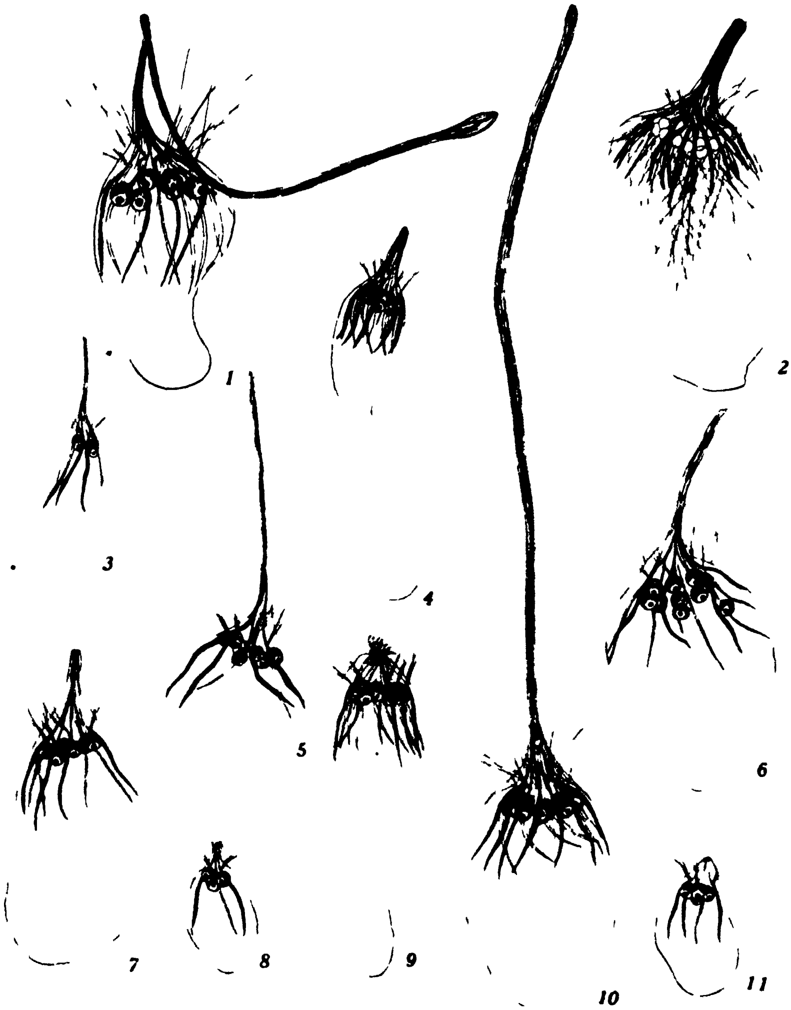

Fig. B. Extension and retraction of the proboscis in *Proboscidiella multinucleata.* × 325. 1. Seven-celled somatella with extended proboscis distended distally. 2. Fourteen-celled somatella with contracted proboscis, showing retractor fibers branching distally and extending beyond the axostyles. 3. Small two-celled somatella with exposed axostylar fibrils at the tip. 4. Ten-celled somatella with body contracted into the shape of a dumb-bell. Proboscis much retracted. 5. Seven-celled somatella with body contracted to a spherical shape and proboscis much extended. 6. Ten-celled somatella with biconical body and proboscis partially extended and capped at the tip. 7. Six-celled somatella with truncate proboscis and fibrils massed distally, and ellipsoidal body. 8. Small three-celled somatella with spreading fibrillar tip to the short proboscis. 9. Ten-celled somatella with proboscis completely retracted and extended fibrils spreading in a brush at the apex of the body which is contracted in a slender posterior lobe. 10. Eleven-celled somatella with greatly extended proboscis and broadly ellipsoidal body. 11 Small four-celled somatella with retracted proboscis.

The irregularity in numbers is due to the unequal fissions of the body (fig. C), and the predominance of even numbers to coincident mitosis of the component nuclei. There is an absence of a continuous integrated fibrillar system and a concurrent irregularity in nuclear distribution.

The *neuromotor* system consists of units each associated with a single nucleus but not interconnected with its neighbors as are the flagellar units in *Oxymonas* and the nucleus-flagellar complexes of *Giardia.* The nearest approach to an integration is the association of the anterior axostylar fibers in the proboscis.

Each unit consists of the following parts, the centrosome (fig. A-1, *cent.*), rhizoplast (*rhiz.*), blepharoplast, the single primary (*pr. fl.*) and the pair of secondary flagella (*sec. fl.*), axostyle (*ax.*), anterior axostyle (*ant. ax.*) spreading into the proboscis fibers (*prob. fib.*), and the retractor fibers (*retr. fib.*). The centrosome lies directly on the anterior face of the nuclear membrane. From it there passes anteriorly a short, delicate rhizoplast to the blepharoplast bar. This is a heavily stained, short, more or less horizontal bar, from whose proximal end, constituting the primary blepharoplast (*pr. bleph.*), arises the single primary flagellum, while from the distal end, the secondary blepharoplast (*sec. bleph.*), emerges the pair of equal, secondary flagella. These are often adherent or twisted together, and appear to be of somewhat heavier caliber than the primary flagellum. The flagella form a ring around the base of the proboscis. They are difficult to find, are relatively small and feebly developed, as though displaced not only in location but also in function by the proboscis. From the primary blepharoplast the axostyle continues posteriorly somewhat near the periphery of the body for nearly half the length of the body in the elongated form (fig. A-2). Immediately behind the blepharoplast it expands abruptly into a band about four times as wide as it is thick and tapers gradually to a fine point distally. In the fully contracted individual with the proboscis retracted and body foreshortened, the axostyles reach the posterior end and may even project a short distance (pl. 31, fig. 2). From the primary blepharoplast there passes anteriorly, as a continuation of the axostyle, what we have called the anterior axostyle (fig. A, *ant. ax.*), a siderophile strand which diverges into separate fibrils which combine with others of like origin from other neuromotor units in the axial bundle of proboscis fibers (*prob. fib.*). Extending posteriorly from the proboscis and radiating beneath the pellicle in the anterior end of the body are a number of fine fibrils of the same appearance as the single fibrils in the proboscis. These extend posteriorly as far as, or even beyond the axostyles, and frequently bifurcate. They lie in the periphery near the pellicle and function as retractor fibers (*retr. fib.*).

The number of neuromotor units, as a rule, is the same as the number of nuclei. In some cases, however, it is greater. For example, a mononucleate individual (pl. 31, fig. 6) was found to have four blepharoplasts, four axostyles, and four flagellar groups. In this case there had evidently been two divisions of the primary neuromotor unit without accompanying nuclear division. In one case (pl. 32, fig. 11) we found no less than thirteen neuromotor units with only a single nucleus. In several other cases we have found supernumerary neuromotor units, one, two, or three in excess of the number of nuclei. These are doubtless due to the division of a like number of primary neuromotor units with the outgrowth of a pair of new axostyles and subsequent disappearance of the old one. These units may thus be merely promitotic phases. There is no evidence in this genus of the regular and normal increase of neuromotor units beyond the number of nuclei such as occurs in *Stephanonympha*, although the frequency of occurrence of supernumerary units in our material may be indicative of a tendency in that direction.

The *proboscis* (fig. A-1, 2, *prob.*) is an organ of the body as a whole. It is formed at the anterior end above the level of the flagellar ring. This region, in the fully contracted phase (pl. 31, fig. 2), forms a low cone whose altitude is equal to its base. Its pellicle must be extremely mobile and elastic to provide for its elevation in a cylinder of uniform caliber whose length above the flagellar ring may be nearly three times that of the body. The proboscis contains an axial bundle of crowded fibers (fig. A, *prob. fib.*) enclosed within the pellicle, which forms the proboscis sheath (*prob. sh.*). Its anterior end may be flared out like a bulb (fig. B-1) or cupped (fig. B-8). In some cases of retraction the fibrils are seen to be twisted in a clockwise spiral within the proboscis. In individuals sectioned *in situ* we find the tip of the axostyle spread out, or cupped, or twisted against the epithelial layer. It is very evident that this is a very mobile, powerful organ of attachment capable of a great variety of movements and probably of local contraction, especially near the tip. It functions as a unit though it contains fibrils from many neuromotor units between which we can not demonstrate fibrillar interconnections. It is possible that such connections might occur within the axial proboscis fibers, but this cannot be determined.

BINARY FISSION

Binary fission in *Proboscidiella* occurs without any evidence of any fixed or definite relation either to mitosis or to the number of cells in the somatella. This is very evident when one inspects large numbers of individuals. We have not seen mitosis, beyond a few early prophases, in this genus as yet, although our material is considerable. On the other hand, the instances of binary fission in various stages of the process are numerous. In a few cases this fission proceeds when the number of cells is small as, for example, in a three-celled somatella (fig. C-2) where the fission results in a one- and a two-celled individual. We have not seen fission in a two-celled somatella. In some instances

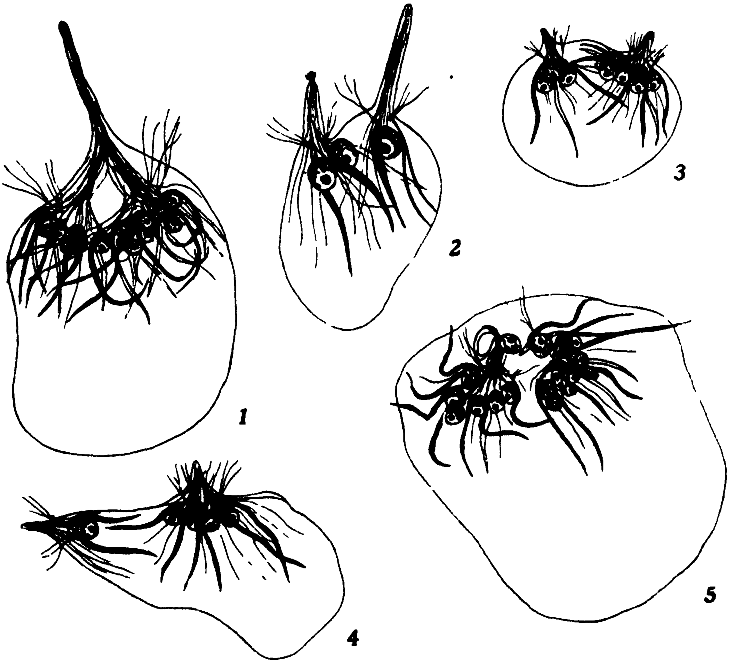

Fig. C. Binary fission (plasmotomy) in *Proboscidiella multinucleata*. × 450. 1. Twenty-celled somatella dividing into two ten-celled daughters by segregation of nuclei into two groups and splitting of axostylar fibrils into two groups distally towards the apex. 2. Three-celled somatella dividing into unequal daughters, one- and two-celled respectively. A fully separated proboscis on each daughter exhibiting independent behavior. 3. Twelve-celled somatella dividing unequally into a three- and a nine-celled daughter. Proboscis on each equally contracted. 4. Eight-celled somatella dividing into a two- and a six-celled daughter. Proboscis in each similarly contracted. 5. Seventeen-celled somatella dividing into two unequal daughters, of seven and ten cells respectively. One proboscis contracted more than the other.

the division is equal as in plate 32, figure 14, where two four-celled daughters are forming, or in figure C-1, where two ten-celled ones are beginning to separate. In other instances, the divisions result in unequal numbers of cells in the two daughters with varying degrees of inequality, for example, 1 and 2 (fig. C-2), 2 and 6 (fig. C-4), 3 and 7 (fig. C-3), and 7 and 10 (fig. C-5). In one instance (pl. 32, fig. 15) a somatella with thirty-four cells had nine of them rather loosely aggregated in one proboscis and the remaining twenty-five scattered elsewhere without any other proboscis being formed. This stage might indicate the approach of multiple fission such as we have described (1915) in *Trichomonas augusta*, in which the constituent cells are successively detached from the parent plasmodium individually. On the other hand, it might be a degenerating individual, but the presence of wood particles in the cytoplasm does not support this view. Normal feeding and metabolism is apparently still in progress.

The process of binary fission is initiated at the level of the blepharoplasts (fig. C-1) by a parting of the anterior axostyles which enter the proboscis in two bundles. It is thus morphologically a longitudinal division. This separation presumably progresses anteriorly to the tip and results ultimately in the parting of the proboscis, possibly when retracted (fig. C-5), into two organs. The presence of a number of somatellas with two such structures is evidence that the process of plasmotomy is a relatively slow one. As in *Trichomonas* (Kofoid and Swezy, 1915), the two daughters probably exhibit a high degree of mobility (fig. C-4; pl. 32, fig. 14) but finally part the common cytoplasm which is the only bond uniting them.

ENCYSTMENT

In certain intestinal smears we have found five instances of the encystment of *Proboscidiella* (pl. 32, fig. 9). The cysts are spherical, 45 microns in diameter, with a very thick (2 to 6 microns), homogeneous, slightly staining wall, not uniformly thick in all regions. In the cyst figured there are six cells, with persistent axostyles and dumb-bell-shaped granules, which may be the already divided blepharoplasts. There is no evidence of the proboscis or of the proboscis fibrils. The nuclei are in an early promitotic phase but are so obscured by the wall as not to reveal the details of the process. Near the apical pole the cyst wall is very thin as though this region had been kept open by the activities of the proboscis during the secretion of the wall.

In another instance in which the wall was evidently in the process of formation the proboscis and flagella were still protruding through the wall at the apical pole. Similar stages are occasionally seen in other flagellates of the termites. They suggest that the process of encystment has not attained, either in the completeness of the process or the consistency of the cyst wall, the high degree of protective efficiency of the cysts of other flagellates; for example, those of the vertebrates.

ORGANIZATION AND INTEGRATION

Certain features of this process are of considerable biological interest. In the first place, the body is strikingly of the same general form as that of *Oxymonas* (Kofoid and Swezy, 1925), the ancestral form from which *Proboscidiella* may well have had its evolutionary origin. Its form, behavior, and binary fission have changed little, if at all. In the second place, the number of nuclei and attendant neuromotor units does not seem to be of much consequence in modifying either structure, reproduction (binary fission), or behavior. In the third place, the integration, structure and behavior is accomplished by the association of the neuromotor fibrillar complexes in the proboscis. The individual figured on plate 32, figure 15, is instructive on this point. The unitary nature is preserved only in the single proboscis in which the anterior axostyles are associated in a close bundle. Elsewhere in this somatella the individual cells are scattered without apparent coördination in either body structure or function. It is to be noted in this connection that no interblepharoplast rhizoplast, such as occurs in *Oxymonas,* has been found in *Proboscidiella.*

It is evident that the integrating bonds, whatever they may be, between nucleus and neuromotor system on the one hand, and between the component nuclei and systems on the other, must be rather weak and easily disrupted or rearranged in this genus.

BIONOMICS

This organism is abundant in the intestine and rectum of its host. It is found attached in greater numbers in the anterior part of the intestine where it occurs with the proboscis thrust through the closely enveloping coat of bacteria which, in closely packed vertical ranks, cover the epithelium with an uninterrupted coating. The tip of the proboscis is often flattened, cupped, or twisted against the cells (pl. 32, figs. 10, 13).

The cytoplasm of this flagellate is often crowded with wood chips, sometimes of large size (pl. 32, fig. 8), or with spheroidal granules in vacuoles (pl. 31, fig. 3), or with a few siderophile droplets (pl. 31, fig. 7) indicating that the cellulose may be appropriated by these organisms. In one instance (fig. D) the posterior end of the body is spread out horizontally in an amoeboid fashion over a wood sliver as long as the normal length of the individual. One process, at the right, is creeping over the end of the piece, and two, at the left, are pushing out toward the other end, while the central part has completely enveloped the shaft of the piece. This pseudopodial type of feeding is not unlike that described by one of us (Swezy, 1923) in *Trichonympha*. Although the surface of the body is generally covered (pl. 31, fig. 5) with a dense coat of vertically attached bacteria, we do not find that the organism directly feeds upon them, or has bacteria-filled food vacuoles. Occasionally one of the smaller flagellates found on the host is included (fig. D).

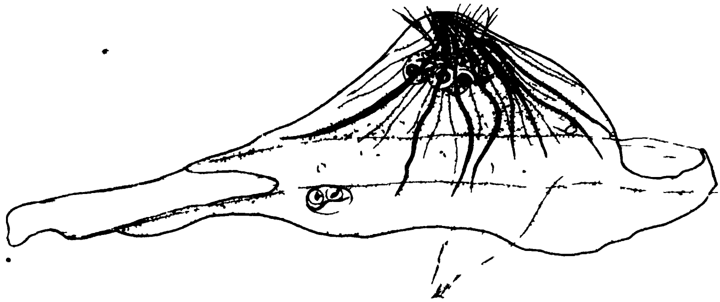

Fig. D. *Proboscidiella multinucleata* ingesting an elongated fragment of wood by the extension of a pseudopodium around the end at the right and the main shaft, with two pseudopodia extending along the shaft to the left. A small flagellate apparently included in the cytoplasm below the shaft. × 875.

RELATIONSHIPS AND EVOLUTIONARY ORIGIN

Proboscidiella is composed of an indefinite but relatively small number of cells of the *Monocercomonas* type each with a neuromotor complex of centrosome, rhizoplast, blepharoplast, three flagella, and axostyle. It adds to this the organ functioning as a proboscis, containing the anterior axostyles of the constituent units. This feature at once allies it to *Oxymonas*, which has the proboscis, and the *Monocercomonas* neuromotor complex, duplicated except for the axostyle. *Proboscidiella* has been derived from *Oxymonas* by a multiplication of the cellular units, the synchronizing of nuclear and neuromotor multiplication, and the preservation of their common unitary state and unitary connection. A single nucleus is accompanied by a single neuromotor system as a rule. In some instances, however, the tendency of the neuromotor system to outstrip the nucleus in the rate of duplication still occurs in *Proboscidiella,* as shown in mononucleate individuals with multiple neuromotor systems including axostyles (pl. 32, fig. 11). Species of *Oxymonas* occur in the termites of the Philippine Islands, though we have not found them in the host of *Proboscidiella.* On the other hand, no species of *Proboscidiella* has as yet been found in American termites.

GENERIC CHARACTERS

Proboscidiella is a polymastigote flagellate with one to many nuclei, each with a neuromotor system with a primary and two secondary anterior flagella, an axostyle, an anterior axostylar process, and nucleus with a large karyosome in halo. Each individual has a single extensile and retractile proboscis containing the anterior axostylar process of each neuromotor system. Equal or unequal binary fission occurs without concurrent or associated antecedent mitosis. Type species, *Proboscidiella multinucleata* Kofoid and Swezy from *Planocryptotermes nocens* Light from Manila, P. I.

SUMMARY

Proboscidiella multinucleata occurs in the anterior part of the intestine of *Planocryptotermes nocens,* attached to the wall by its proboscis. It is a multinucleate polymastigate flagellate with one to thirty-seven cells with their nuclei grouped anteriorly at one level. The nucleus has a posteriorly located karyosome in a clear halo, and a well-defined chromatin net. The neuromotor system consists of an anterior centrosome on the nuclear membrane, rhizoplast, bar-shaped blepharoplast, with a primary granule giving rise to the primary anterior flagellum, axostyle and an anterior axostylar process, and a secondary or distal granule giving rise to the two secondary flagella. Each somatella regardless of the number of nuclei has a single extensile and retractile proboscis to whose mobile tip all the single anterior axostyles extend. Siderophile tubular structures, possibly vestigial sleeves, such as occur around the axostyle of *Oxymonas,* are found in some individuals. Wood chips are ingested by the amoeboid action of the posterior end. Binary fission of the body occurs with equal or unequal distribution of the constituent nuclei. Supernumerary neuromotor systems have been observed in mononucleate individuals. The evolutionary derivation of *Proboscidiella* from an *Oxymonas*-like ancestor is suggested by both morphology and behavior.

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Zoological Laboratory,
University of California.

Transmitted October 31, 1925.

EXPLANATION OF PLATES

All figures are camera lucida drawings of *Proboscidiella multinucleata* gen. nov., sp. nov. from *Planocryptotermes nocens* Light from Manila from intestinal smears fixed in Schaudinn's fluid and stained in iron-haematoxylin. × 875 unless otherwise stated.

PLATE 31

Fig. 1. Lateral view of six-celled somatella with proboscis partially extended, two tubular bodies or vestigial sleeves, and particles of wood in the cytoplasm.

Fig. 2. The same of a contracted eight-celled somatella with the proboscis withdrawn, axostyles protruding posteriorly and circlet of flagella around the base of low proboscis cone.

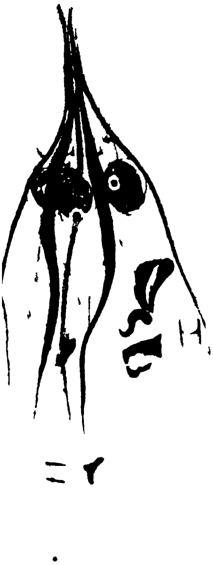
Fig. 3. The same of a six-celled somatella with numerous pale spheres in vacuoles in the cytoplasm. Three blepharoplasts and three sets of anterior flagella drawn.

Fig. 4. Neuromotor system of a seven-celled somatella with contracting proboscis with cup-shaped tip. Ten neuromotor units, the three nuclei at the right appear to have two each, probably an early promitotic state. Anterior axostyles with somewhat dispersed fibrils.

Fig. 5. Lateral view of a six-celled somatella with spirally twisted, partially contracted proboscis. Body covered with bacteria. Five vestigial tubes in the cytoplasm.

Fig. 6. Mononucleate somatella with slender extended proboscis, and four neuromotor units. The nuclear rhizoplasts could not be determined.

Fig. 7. Twelve-celled somatella with several supernumerary neuromotor units (axostyles), two small tubules and siderophile droplets in the cytoplasm, and contracting proboscis.



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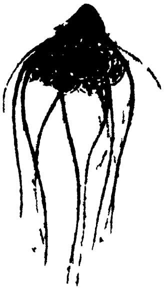

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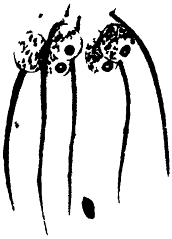

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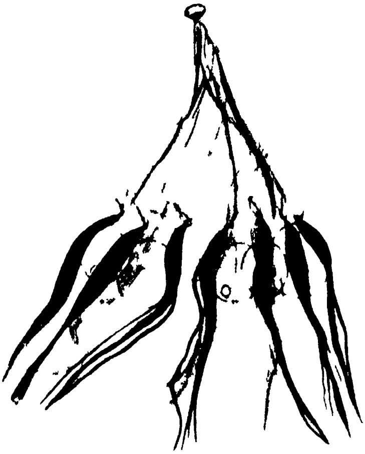

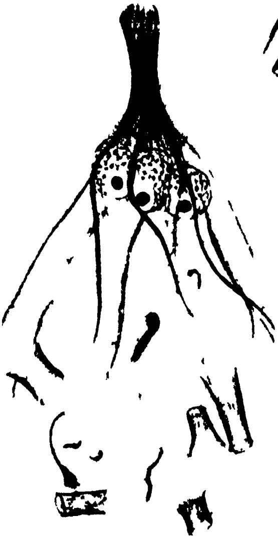

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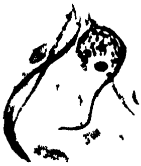

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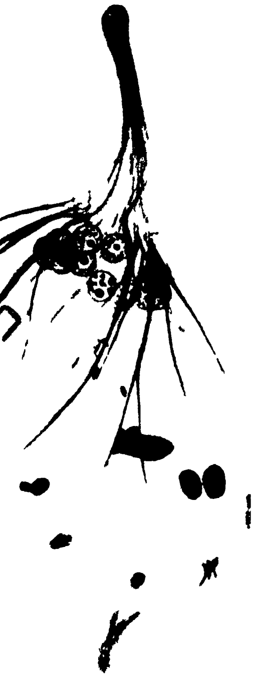

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PLATE 32

Proboscidiella multinucleata gen. nov., sp. nov.

Fig. 8. Flattened somatella of nine cells with thirteen axostyles, showing their union in the proboscis.

Fig. 9. Encysted six-celled somatella with nuclei in prophase and seven axostyles. Note thin area in cyst wall near the apical pole.

Fig. 10. Cross-section of the intestinal wall of *Planocryptotermes nocens* showing *Proboscidiella in situ* with proboscis thrust through the layer of bacteria and attached by its tip to the surface of the epithelium. Note the expanded, cup-shaped tip.

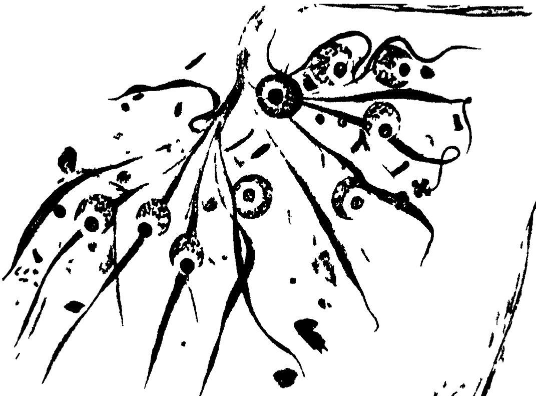
Fig. 11. Mononucleate somatella with fourteen neuromotor units and anterior axostyles combined in one proboscis.

Fig. 12. Two nuclei with their attendant neuromotor systems. Karyosome with spoke radii and central clear area with a centriole. Neuromotor system with centrosome on nuclear membrane, nuclear rhizoplast, blepharoplast bar with primary and secondary blepharoplasts at its proximal and distal ends respectively, single primary and pair of secondary flagella, broad axostyle, and slender anterior axostyle. No fibrillar connection between the two systems. × 1800.

Fig. 13. Tips of severed *Proboscidiella in situ* attached to the intestinal wall from sections. × 1800.

Fig. 14. Eight-celled somatella in equal binary fission. Two small vestigial tubes in the cytoplasm.

Fig. 15. Thirty-four-celled somatella, with one proboscis with nine component units. The remaining twenty-five are without relation to any proboscis, suggesting multiple fission.



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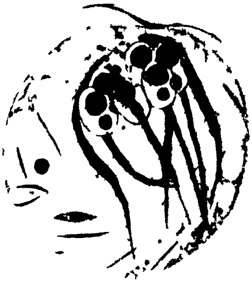

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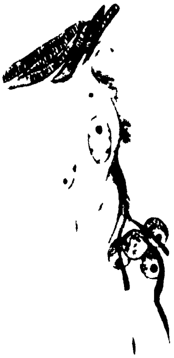

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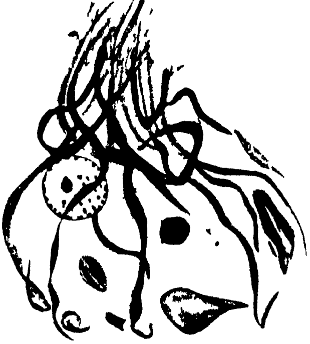

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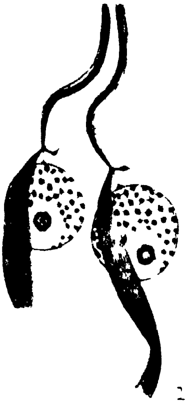

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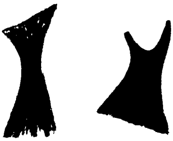

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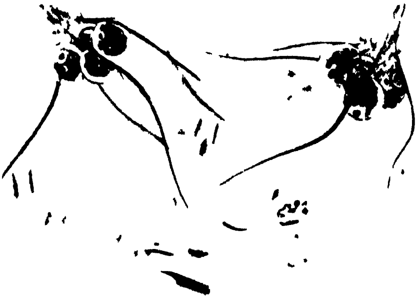

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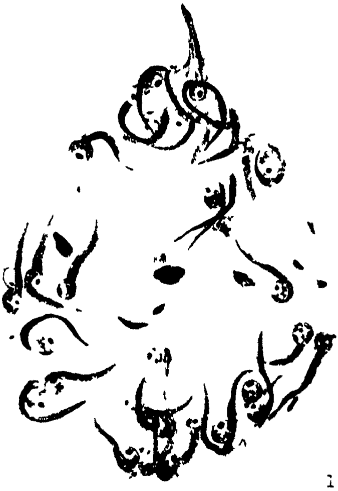

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ON THE CESTODE GENUS DIPYLIDIUM FROM CATS AND DOGS

BY

THERESA MARIE MILLZNER

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ON THE CESTODE GENUS DIPYLIDIUM FROM CATS AND DOGS

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THERESA MARIE MILLZNER

Seventeen species of *Dipylidium* have been described, 8 of which were from dogs or cats, but up to 1917 only one, *Dipylidium caninum,* had been reported from the United States. In 1917, another species, *Dipylidium sexcoronatum,* was reported for the first time from the United States by M. C. Hall. He found it to be quite common in dogs from Detroit and suspected that many double-pored tapeworms reported as *D. caninum* were in reality *D. sexcoronatum.* In a later paper (1918) he mentions that some of the specimens he examined, while superficially resembling *D. sexcoronatum,* appeared to have only 5 rows of hooks and he suggested the need of further study in order to determine "whether *D. sexcoronatum* has sometimes 5 rows of hooks, as well as 6 rows, or whether this material belongs to a new species."

Several years ago, while examining a kitten for parasites, I found a number of tapeworms belonging to the genus *Dipylidium.* Superficially they resembled *Dipylidium caninum* although they were much smaller and the rostellum was armed with 5 instead of 4 rows of hooks. Several of these worms were mounted and preserved but no further effort was made to identify them. In 1924, during the course of some other work, several dogs and cats were examined for *Dipylidium* and a number of worms obtained. These worms showed extreme variations, some being characteristically short, others long, some with broad cucumber-seed-like segments, others with long narrow rectangular segments. Examination of these worms and comparison with the descriptions of *Dipylidium* by Hall (1919) failed to allocate them definitely, although they resembled *Dipylidium sexcoronatum* more than any of the other species of *Dipylidium* which he describes. The present work was undertaken in order to determine definitely whether or not this material represented only variations of *Dipylidium sexcoronatum* or whether these worms belonged to several distinct species of *Dipylidium.*

HISTORICAL

The genus *Dipylidium* of Leuckart (1863) is composed of tapeworms belonging to the subfamily Dipylidiinae (Stiles, 1896) of the family Hymenolepididae (Railliet and Henry, 1909). The worms in this genus have the following characteristics. The genital pores are double, one being on each side of the segment. At each genital pore is the opening of the vagina and the cirrus pouch. All the genital organs with the exception of the uterus are double in each segment. The uterus is reticular at first but later breaks up into a number of egg capsules in each of which lies one or more double-shelled eggs. The rostellum is retractile and bears several alternating rows of rose-thorn-shaped hooks, each of which has a small discoid base.

The type species of the genus is *Dipylidium caninum,* first described by Linnaeus in 1758 under the name of *Taenia canina.* Although found in both dogs and cats, the worms from the two hosts were for a long time thought to be different and were repeatedly described under different names, such as *Taenia cucumerina* and *Taenia elliptica,* because the worms found in the cat were regularly smaller than those found in the dog.

Sonsino (1889) found a tapeworm in the small intestine of *Megalotis cerdo* in Egypt and named it *Taenia echinorhyncoides.* A few years later this form was studied by Diamare who placed it in the genus *Dipylidium.* Diamare (1892) described a tapeworm belonging to this genus which he had obtained from the intestine of a domestic cat in Naples and which had also been found by Pasquale in the intestine of a cat at Alexandria, Egypt. He named this *Dipylidium trinchesi.* In the same year Pasquale found a different species of *Dipylidium* in the intestine of a cat at Alexandria. This species was also described by Diamare (1893*a*) as *Dipylidium pasqualei* after its discoverer. Diamare (1893*b*) in an article on the four species of *Dipylidium* then known redescribed the above species and in addition mentioned two other doubtful species, *Dipylidium genettae* (Gervais) (1847) and *Dipylidium monticellii* Diamare (1893*b*). *Dipylidium genettae* is based on a very imperfect description and two schematic drawings by Gervais, who named it *Halysis genettae.* The host was *Viverra genetta.* The drawings and description were so imperfect that Diamare was unable even to decide definitely whether or not it belonged in the genus *Dipylidium*. *Dipylidium monticellii* was described on the basis of a specimen in the collection of the British Museum at London. This specimen had been collected by an expedition to the Euphrates, but the host of this species is unknown.

The next species to be described was *Dipylidium gervaisi,* which was found by Setti (1895) in a *Genetta tigrina* from Eritrea.

Two years later von Ratz (1897) described a tapeworm which he had found in the small intestine of cats in Hungary. This worm resembled *Dipylidium pasqualei* more than it did any other previously described species, but it differed from *D. pasqualei* in many important points. Von Ratz named this form *Dipylidium chyzeri.*

Lühe (1898) described two species of *Dipylidium, D. triseriale* and *D. monoöphoron*. Both were very small, 25 and 10 mm. in length, respectively. Both were found in the small intestine of civet cats, *Viverra civetta* in Tunis.

·Von Ratz (1900) described two more species of *Dipylidium*. One, *D. oerleyi,* was found in cats at Budapest, Hungary; the other, *D. sexcoronatum,* was originally described from specimens obtained from dogs at Budapest. Since then *D. sexcoronatum* has been reported by Hall (1917) from dogs from Bethesda, Maryland, and Detroit, Michigan. He also mentions (1923) its occurrence in cats from Massachusetts. Sondhi (1923) reported it from dogs in the Punjab and also from dogs in England, so it appears that the distribution of this worm is practically cosmopolitan.

Fuhrmann (1906) described a tapeworm, *Dipylidium avicola,* which he had found in a bird, *Gyps kolbi,* from South Africa. This was the first adult *Dipylidium* found in a host other than mammal. A second avian *Dipylidium, D. columbae,* was described by Fuhrmann (1909) from *Columba sp.* from Egypt.

Hungerbühler (1910) described as *Dipylidium zschokkei* a worm which he had obtained from *Cynictis penicillata* in Sekuma (South Africa).

Beddard (1913), while examining the small intestine of a Dongolian genet, *Genetta dongolana,* which had died at the Zoological Gardens at London, found five small tapeworms which he named *Dipylidium dongolense.*

Rodriguez Lopez-Neyra and Munoz Medina (1921) published the description of a new species, *Dipylidium quinquecoronatum,* which they had found in the small intestine of domestic cats at Granada, Spain. This species somewhat resembled *Dipylidium trinchesi*, which they also found in cats at Granada.

Sondhi (1923) published a paper on the tapeworm parasites of dogs in the Punjab in which he described a new species, *Dipylidium walkeri.*

MATERIAL AND METHODS

For the present work 28 dogs and 30 cats, all obtained in Oakland, Alameda, and Berkeley, were examined for *Dipylidium.* The animals were killed by illuminating gas and dissected while still warm. The intestines were removed and slit open for their entire length. The tapeworms were loosened from their attachments by carefully brushing them with a camel's hair brush dipped in normal salt solution. They were then placed in albumen water preparatory to fixing. Albumen water was used instead of normal salt solution because it was found that the worms kept alive for hours in water to which one-third of its volume of white of egg had been added, while they died and began to disintegrate after a rather short time in normal salt solution. All the worms were removed from each animal and preserved.

A number of fixing fluids were tried, but the only one that satisfactorily answered the requirements (1) of rapid fixation without contraction of the worm, and (2) good preservation as shown by subsequent staining, was an acetic sublimate mixture. This was made by adding 10 per cent of glacial acetic acid to a saturated aqueous solution of corrosive sublimate. In fixing, the worms were taken one at a time from the albumen water, rinsed in warm normal salt solution to remove excess albumen water, and laid on a warm glass plate. A camel's hair brush dipped in warm (50°) acetic sublimate was then used to straighten the worms. This gave fixation with the worms absolutely flat and without contraction. As soon as the worm was straight, more fixing fluid was added. When the worm became opaque, usually after 10 to 30 seconds, it was taken off the plate and put into a vessel of warm fixing fluid.

After half an hour in the fixing fluid, the worms were quickly rinsed in water to remove excess sublimate, and then washed in two changes each of 35 per cent and 50 per cent alcohol. They were then preserved in 70 per cent alcohol.

A number of different methods of mounting were tried with *Dipylidium* taken from other animals before deciding which method to follow. Direct mounts in glycerine as well as mounts made by placing the worms in dilute (10 per cent) glycerine, allowing it to concentrate and then mounting in glycerine jelly, were found to be extremely unsatisfactory. Mounts made by staining for a few minutes in dilute picro-carmine (Ranvier's), washing in acidulated water, dehydrating, clearing and mounting in balsam, gave fairly satisfactory but not uniform results. Borax carmine was found to be unsatisfactory. Delafield's and Ehrlich's haematoxylin both gave satisfactory results but the staining was not sufficiently uniform and showed too much variation in the depth of staining of different segments of even the same worm to be of much value when every worm was to be mounted. The most satisfactory and uniform results were obtained with dilute alum carmine. Progressive staining with very dilute solutions was found to give much clearer pictures than regressive staining in which the worms were overstained and then partly decolorized with acid alcohol.

The following technic was found to be the most satisfactory and was uniformly used. After fixing the worms for one half-hour in acetic sublimate, they were washed in alcohol. Excess sublimate was then removed by the use of dilute alcoholic iodine solution. The iodine was removed by adding a few drops of sodium thiosulphate solution to the water used for final washing. The worms were stained 24 hours in dilute alum carmine made by adding 15 drops of alum carmine solution to 20 c.c. distilled water. The stained worms were washed in distilled water, dehydrated gradually in alcohol, cleared in creosote, and mounted in balsam.

RESULTS

Of the 28 dogs examined, 25, or 89 per cent, contained *Dipylidium*. The largest number of worms present in any dog was 71; the next largest 52; and the smallest 1. A total of 385 worms was found in the 25 positive dogs, making an average of 15.4 worms per dog. On examining the worms after they had been stained and mounted, it was noted with great surprise that only 3 of them were *Dipylidium caninum*. Of the remaining 382 worms, one was *Dipylidium sexcoronatum*, while the others belong to two new species, *Dipylidium crassum and D. gracile*, which are described in detail farther on.

The number of worms found in each dog was:

	D. caninum	D. sexcoronatum	D. crassum, sp. nov.	D. gracile sp. nov.
Dog 1	3	—	—	—
Dog 2	—	—	12	—
Dog 3	—	—	—	5
Dog 4	—	—	—	1
Dog 5	—	—	—	3
Dog 6	—	—	9	—
Dog 7	—	—	4	—
Dog 8	—	—	17	—
Dog 9	—	—	11	—
Dog 10	—	—	16	—
Dog 11	—	—	6	—
Dog 12	—	—	13	—
Dog 13	—	—	—	52
Dog 14	—	—	—	6
Dog 15	—	—	—	22
Dog 16	—	—	—	8
Dog 17	—	—	—	16
Dog 18	—	—	—	34
Dog 19	—	—	—	12
Dog 20	—		5	—
Dog 21	—	—	—	21
Dog 22	—	—	—	17
Dog 23	—	-	-	18
Dog 24	—	—	—	71
Dog 25	—	1	—	—

The total number and percentage of each species was:

D. sexcoronatum	1 worm	0 2%
D. caninum	3 worms	0 8%
D. crassum, sp. nov.	95 worms	25%
D. gracile, sp. nov.	286 worms	74%

Of the 30 cats examined, 25, or 83 per cent, contained one or more *Dipylidium.* The largest number of worms present in any cat was 314; the next largest 197; the smallest 1. A total of 845 worms was found in the 25 positive cats, making an average of 33.8 worms per cat. On examining the stained and mounted worms, it was found that not even a single specimen of *Dipylidium caninum* was present. There were 24 *Dipylidium sexcoronatum.* The remaining worms belonged to 4 new species, *Dipylidium gracile, D. compactum, D. diffusum,* and *D. longulum.* Of these, *D. gracile* was also found in dogs. Detailed descriptions of these new species are given later.

The number of worms found in each of the 25 positive cats was:

	D. sexcoronatum	D. gracile sp nov.	D. compactum sp. nov.	D. diffusum sp. nov.	D. longulum sp. nov.
Cat 1	—	18	51	—	—
Cat 2	-	27	—	—	—
Cat 3	—	—	18	—	—
Cat 4	—	2	—	—	-
Cat 5	—	135	79	45	55
Cat 6	4	60	114	13	6
Cat 7	6	—	—	—	—
Cat 8	-	11	—	—	—
Cat 9		7	—	—	—
Cat 10	—	3	—	—	—
Cat 11	5	-	—	—	—
Cat 12	-	—	—	—	10
Cat 13	—	2	—	1	11
Cat 14		14	16	—	2
Cat 15	-	38	—	—	—
Cat 16	—		5	-	—
Cat 17	8	—	—	-	—
Cat 18	-	4	—	—	—
Cat 19		—	7	—	—
Cat 20	-	9	—	—	—
Cat 21	1	—	-	—	—
Cat 22	—	12	—	—	—
Cat 23	—	—	3	—	—
Cat 24			27	—	—
Cat 25	—	-	16	—	—

The total number and percentage of each species was:

D. sexcoronatum	24 worms	3%
D. gracile, sp. nov.	342 worms	40%
D. compactum, sp. nov.	336 worms	40%
D. diffusum, sp. nov.	59 worms	7%
D. longulum, sp. nov.	84 worms	10%

DETAILED DESCRIPTION OF SPECIES

These descriptions are in all cases based on our material. In some particulars, therefore, our description of the two species previously known, *D. caninum* and *D. sexcoronatum*, differs from Hall (1918), perhaps because his data may have been based in part on some features of the species here described for the first time.

Dipylidium caninum (Linnaeus) (1758) Railliet (1892)

The scolex is small and rhomboidal in shape. It measures 0.365 mm. in length by 0.327 mm. in width. The four suckers are large and prominent. They are oval, 0.165 mm. by 0.150 mm. The rostellum is conical. It is usually retracted and in this condition measures 0.060 mm. in length; it is 0.100 mm. wide at the base. The rostellum bears four rows of rose-thorn-shaped hooks. The hooks in the anterior row are the largest, 0.012 mm. long; while those in the posterior row are the smallest, 0.007 mm. long. There are 16 to 25 hooks in each row. The neck is narrower than the scolex and very long. It is not scalloped. The segments are at first narrow, wider than long, and indistinct. They soon become distinctly marked off and longer than wide but remain very narrow for 1 to 2 cm. The genital primordia first appear in about the 45th segment as darkly staining masses. The mature segments are 3 mm. long and 1.25 mm. wide. The ripe segments are 12 mm. long and 2.5 to 2.8 mm. wide. Both the mature and ripe segments have smooth convex lateral margins. The genital pores lie slightly posterior to the middle of the segment. The longitudinal excretory canals are narrow but distinct. The entire strobila reaches a length of 50 cm. and a width of 2.5 to 3 mm.

The cirrus pouch is pyriform, 0.170 mm. in length and 0.060 mm. in width. It may extend to the longitudinal excretory canal but usually remains lateral to it. The cirrus pouch slants somewhat anteriorly. The vas deferens passes a short distance medially and anteriorly after leaving the cirrus pouch, and then forms a much coiled mass. The testes are spherical, and 0.055 mm. in diameter. They number 110 to 175 and occupy most of the available space medial to the excretory canals.

The vagina opens posterior to the cirrus pouch. In young segments the vagina is rather tortuous, but in older ones it becomes almost straight. It passes medially and anteriorly until it reaches the longitudinal excretory canal. It then bends posteriorly in a rather sharp curve. The two lobes of each ovary are separated by the vagina which passes between them. The lateral ovarian lobe is slightly smaller than the median ovarian lobe. The ovarian lobes are definitely lobulated and appear moderately compact. Each entire ovary is roughly elliptical in shape and about 0.430 mm. long by 0.275 mm. wide. The compactly lobulated vitellaria are reniform to elliptical in shape. One lies a short distance posterior to each ovary. Each measures 0.160

by 0.165 mm. and is smaller than either ovarian lobe. A small but distinct shell gland lies between each ovary and vitellarium. No seminal receptacle is present. The egg capsules extend lateral to the longitudinal excretory canals and are closely packed, leaving only a small portion of the anterior part of the segment free. Each egg capsule contains from 4 to 20 eggs. The eggs are spherical and 0.050 mm. in diameter.

Host.—Dog.

Dipylidium sexcoronatum von Ratz (1900)

The scolex is oval to conical in shape. It is 0.365 mm. long and 0.350 mm. wide. The suckers are prominent and four in number. They are oval, 0.185 mm. long by 0.160 mm. wide. The rostellum is conical and is usually retracted. It is 0.065 mm. long by 0.115 mm. wide at the base. It is armed with 6, occasionally 7 rows of rose-thorn-shaped hooks. The hooks near the tip are larger, 0.011 mm., than those near the base, 0.004 mm. There are 20 to 25 hooks in each row, the number increasing near the base. The neck is short and almost as broad as the scolex. It is irregularly scalloped at the edges. Posteriorly the scallops become deeper and finally complete, cutting off the anterior segments. The anterior segments are broad and short. The older segments gradually become longer so that they are almost square in the region of the mature segments. The ripe segments are several times longer than wide. The mature segments are 1.5 mm. long and 1.3 mm. wide. The ripe segments are 10 mm. long and 2 mm. wide. All the segments have a more or less irregular lateral margin. The posterior end of each segment, specially in the more anterior ones, forms a prominent collar over the anterior end of the following segment. The genital primordia appear between the 15th and the 30th segment. Both the longitudinal and transverse excretory canals are usually prominent, even in young segments. The entire strobila reaches a length of 10 to 30 cm. and a maximum width of 2 mm.

The genital pores are two in number, situated at about the middle of the segment. The cirrus pouch is pyriform in young segments. As the segments grow older, the cirrus pouch becomes constricted medially, thus becoming hour-glass-shaped in the mature segments. Later this constriction disappears, so that the cirrus pouch becomes pyriform again in the ripe segments. In the mature segments it measures 0.290 mm. long by 0.060 mm. wide. The cirrus pouch extends to but does not cross the longitudinal excretory canal. The vas deferens forms a coiled mass medial to the cirrus pouch. The testes are spherical and 0.055 mm. in diameter. They are about 150 to 175 in number and occupy most of the space between the longitudinal excretory canals that is not occupied by other reproductive organs. The testes are more numerous in the posterior portion of the segment and leave a clear space anteriorly and between the ovaries.

The vagina opens posterior to the cirrus pouch. It runs directly medially or slightly anteriorly as far as the longitudinal excretory canal and then gradually curves posteriorly between the two ovarian lobes. Each bilobed ovary forms a coarsely lobulated, almost spherical, mass, 0.220 by 0.295 mm. The ovaries lie posterior to the level of the genital pores. The lateral ovarian lobe is slightly smaller than the median ovarian lobe. A coarsely lobulated vitellarium lies a short distance posterior to each ovary. The vitellaria are reniform and measure 0.125 by 0.180 mm. Each is about the size of one of the lateral ovarian lobes and is usually smaller than one of the median ovarian lobes. A small reniform shell gland lies between each ovary and vitellarium. A spindle-shaped seminal receptacle is present. The uterus appears as a fine tubular network in the post-mature segments. The egg capsules fill most of the ripe segments except for the most anterior portions. They extend lateral to the longitudinal excretory canals. Each capsule contains from 2 to 15 eggs. The eggs are spherical and 0.025 mm. in diameter.

Hosts.—Dog and cat.

Dipylidium gracile, sp. nov.

The scolex is rhomboidal to oval in shape and measures 0.410 mm. long and 0.460 mm. wide. The four suckers are fairly prominent and are nearly round. They measure 0.170 by 0.172 mm. in diameter. The rostellum is conical. It is 0.070 mm. in length and 0.120 mm. in width at the base. It bears 5 to 7 rows of rose-thorn-shaped hooks. The hooks are largest near the tip of the rostellum. The hooks of the most anterior row are 0.008 mm. long; those of the posterior row, 0.005 mm. long. Each row consists of from 20 to 30 hooks. The neck is narrower than the scolex, long, and unsegmented. Its margins are not scalloped. The first segments are much wider than long, but, as they grow older rapidly become longer than wide. The posterior end of each segment is wider than the anterior end of the following segment. The genital primordia appear between the 10th and the 20th segment. Both the mature and ripe segments are rectangular in shape and have smooth lateral margins. The mature segments are 3 mm. long and 1 mm. wide. The ripe segments are long and narrow, 10 mm. long by 1.25 mm. wide. The genital pores are posterior to the middle of the segment. The excretory canals are not prominent and are usually made out with difficulty or are not visible. The strobila length varies from 4 to 20 cm., depending chiefly on the number of ripe segments present. The maximum width is 1.25 to 2 mm.

The cirrus pouch extends to or slightly medial to the longitudinal excretory canals. It is directed anteriorly. It is pyriform to cylindrical in shape, 0.255 mm. long and 0.075 mm. wide. The vas deferens forms a coiled mass medial to each cirrus pouch. The testes are numerous, 200 to 250 in each segment. They are spherical and 0.070 mm. in diameter.

Each vagina opens posteriorly to the cirrus pouch, then courses medially and anteriorly in a somewhat sinuous manner. After crossing the longitudinal excretory canal, runs medially a short distance and then usually curves abruptly posteriorly between the ovarian lobes. Each ovary is divided into two lobes by the vagina, the lateral lobe usually being the smaller. The entire ovary forms a globular mass, 0.290 by 0.290 mm. The ovaries are very finely lobulated. In stained specimens their very diffuse and poorly outlined appearance is characteristic. An equally diffuse and finely lobulated vitellarium lies posterior and almost contiguous to each ovary. The vitellarium is reniform to globular in shape and measures 0.165 by 0.220 mm. It is about the size of the lateral ovarian lobe. A narrow elongated seminal receptacle is present. Between the ovary and vitellarium lies a small indistinct shell gland. The net-shaped uterus is fairly distinct in some of the postmature segments. The egg capsules are closely packed and fill most of the space in the ripe segments with the exception of the anterior portion and extreme lateral and posterior margins. Each egg capsule contains from 3 to 10 eggs. The spherical eggs are 0.030 mm. in diameter.

Hosts.—Dog and cat.

Dipylidium compactum, sp. nov.

The scolex is 0.410 mm. in length by 0.420 mm. in width, oval in shape, and terminates conically anteriorly. The suckers are four in number, prominent, and are nearly round, 0.175 by 0.180 mm. The rostellum is conical and is usually retracted. When retracted, it is 0.065 mm. long and 0.100 mm. wide at the base. The rostellum bears 6 rows of rose-thorn-shaped hooks. Those anteriorly are largest, 0.015 mm. long; while those posteriorly are smallest, 0.009 mm. long. There are from 15 to 20 hooks in each row. The neck is short and nearly as broad as the scolex. The sides are scalloped by an incomplete segmentation, which soon becomes complete, thus cutting off the anterior segments. The anterior segments rapidly become elongated, and after a short distance, are longer than wide. The genital primordia appear between the 10th and the 20th segment. Both the mature and ripe segments are rectangular in shape and have smooth or only slightly irregular lateral margins. The posterior end of each segment forms a collar about the smaller anterior end of the following segment. The mature segments are 3 mm. long and 1.5 mm. wide; the ripe segments, 6 mm. long and 2 mm. wide. The genital pores are posterior to the middle of the segment. Both the longitudinal and transverse excretory canals are fairly prominent. The entire strobila is from 5 to 15 cm. long and attains a maximum width of 2 mm.

The pyriform cirrus pouch extends medially and curves anteriorly from the genital pore on each side. The cirrus pouch remains lateral to the longitudinal excretory canal. It is 0.245 mm. long and 0.060 mm. wide. The testes are numerous, 140 to 200 in number, and fill most of the available space medial to the excretory canals. Each testis is 0.065 mm. in diameter. The vas deferens forms a coiled mass medial to each cirrus pouch.

The vagina lies posterior to the cirrus pouch and passes rather tortuously anteriorly and medially from the genital pore as far as the longitudinal excretory canal. It then turns rather sharply and curves posteriorly in a wide curve, passing between the lobes of the ovaries. The ovaries are bilobed. The two ovarian lobes are nearly equal, although the lateral lobe may be somewhat smaller. Each lobe is composed of compact, dark-staining lobules. The entire ovary is nearly globular and measures 0.275 by 0.280 mm. The anterior ends of the ovaries lie slightly anterior to the level of the genital pores. The coarsely lobulated and deeply staining vitellarium lies just posterior to the ovary. It is nearly round, 0.170 by 0.180 mm., and about the size of the lateral ovarian lobe. The seminal receptacle is large, spindle to fusiform in shape, and lies between the ovarian lobes. A small shell gland lies midway between each ovary and vitellarium. The uterus may be indistinctly made out as a fine tubular network in the postmature segments. The egg capsules extend lateral to the longitudinal excretory canals. They occupy most of the segment with the exception of the extreme anterior end. Each egg capsule contains from 2 to 20 globular eggs, each of which is 0.036 mm. in diameter.

Host.—Cat.

Dipylidium diffusum, sp. nov.

The cuboidal to globular scolex is large, measuring 0.450 mm. long by 0.460 mm. wide. Laterally it shows some scalloping. Its light staining appearance is very characteristic. The suckers are four in number, nearly round, and only slightly prominent. They are 0.175 by 0.183 mm. The conical rostellum is 0.075 mm. long by 0.110 mm. wide at the base when retracted. It bears 6, occasionally 7 to 8 rows of rose-thorn-shaped hooks. The anterior hooks are 0.009 mm. long and the posterior hooks 0.005 mm. long. The number of hooks varies from 10 to 20 in each row, those in the posterior rows being the most numerous. The neck is very short and is narrower than the scolex. It is scalloped with incomplete segmentations which become complete almost immediately. The segments are much shorter than broad at first, but soon become trapezoidal and then rectangular. They are a trifle broader posteriorly than anteriorly, and the posterior border of each forms a sort of a collar over the anterior portion of the following segment. The mature segments are 2 mm. long by 1.5 mm. wide. The ripe segments are 5 mm. long by 2.5 mm. wide. Both the mature and ripe segments have somewhat scalloped borders. The genital pores are at or near the middle of each segment. The excretory canals are narrow. They are rather prominent in the mature but are frequently not visible in the ripe segments. The entire strobila attains a length of from 7 to 15 cm. and a maximum width of 2.5 mm.

Each cirrus pouch is pyriform, 0.225 mm. long and 0.070 mm. wide. It extends to but usually does not cross the longitudinal excretory canals. From it, the vas deferens passes medially and anteriorly, forming a coiled mass. The testes are numerous, 160 to 250, and are closely packed between the longitudinal excretory canals, leaving only small areas free at the anterior end of the segment and between the ovaries. Each testis is spherical and 0.070 mm. in diameter.

Each vagina opens posterior to the cirrus pouch and pursues a tortuous course medially and anteriorly as far as the longitudinal excretory canal, where it turns posteriorly in a gentle curve. Each ovary is bilobed and is diffusely lobular. The vagina passes between the ovarian lobes. The lateral ovarian lobe is smaller than the median ovarian lobe. The entire ovary is 0.320 mm. long by 0.315 mm. wide and appears a globular mass. A somewhat more compact, dark-staining, coarsely lobulated reniform vitellarium lies immediately posteriorly from each ovary. It is 0.130 mm. by 0.230 mm. and is as large as the lateral ovarian lobe. The seminal receptacle is narrow and elongated. An indistinct globular shell gland lies between each ovary and vitellarium. In segments midway between mature and ripe, the uterus appears as an indistinct tubular network surrounding the remains of the degenerating testes. The egg capsules are closely packed and extend lateral to the longitudinal excretory canals. Each contains from 3 to 10 eggs. The eggs are spherical and 0.033 mm. in diameter. ,

Host.—Cat.

Dipylidium longulum, sp. nov.

The scolex is rhomboidal to club-shaped. It is 0.410 mm. in length and 0.440 mm. in width. The four suckers are rather prominent and measure 0.170 mm. by 0.175 mm. The rostellum is conical and when retracted measures 0.065 mm. in length by 0.110 mm. in width at the base. The rostellum bears 5 rows of rose-thorn-shaped hooks. The hooks of the anterior row are 0.007 mm. long; those of the posterior row are 0.005 mm. long. There are about 20 hooks in each row. The neck is always narrower than the scolex. It is long and usually unsegmented, although it may show some slight scalloping. The anterior segments are at first wider than long, then rapidly become square, then trapezoidal and longer than wide, and finally rectangular. The genital primordia appear early, usually between the 10th and the 15th segment. The mature and ripe segments are rectangular in shape and have smooth convex lateral margins. The posterior end of each segment is broader than, and forms a sort of a collar over the anterior end of the following segment. The genital pores open slightly posterior to the middle of the segment. The excretory canals are narrow but distinct. The strobila has a total length of from 5 to 12 cm. and a maximum width of about 2.3 mm.

Each cirrus pouch extends medially and anteriorly from the genital pores. The cirrus pouch extends to or remains slightly lateral to the longitudinal excretory canal. Rarely the cirrus pouch may cross the longitudinal excretory canals. It is pyriform in shape and 0.275 mm. long by 0.075 mm. wide. Medial to each cirrus pouch lies the much-coiled vas deferens. The testes occupy practically the entire field medial to the longitudinal excretory canals, leaving only the inter-ovarian field free. There are from 180 to 240 testes in each segment. Each testis is spherical and 0.060 mm. in diameter.

The vagina passes medially and anteriorly from its opening posterior to the cirrus pouch. Usually it is only slightly sinuous. After crossing the longitudinal excretory canals, it curves rather sharply posteriorly between the ovarian lobes. Each ovary is bilobed and composed of dark staining compact lobules. The lateral ovarian lobe is the smaller. The anterior end of each ovary is at the level of the genital pore. Each ovary is roughly spherical, 0.365 mm. by 0.400 mm. Posterior to each ovary and usually in contact with it is an oval vitellarium, 0.160 mm. by 0.240 mm. The vitellarium is smaller than the lateral ovarian lobe. It is coarsely lobulated and dark staining. Between the two ovarian lobes lies a fusiform shaped and distinctly visible seminal receptacle. The shell gland is globular and indistinct. It lies close to the anterior aspect of the vitellarium. The uterus may be distinctly seen in some of the postmature segments as a tubular network between the meshes of which lie the remains of the degenerating testes. The egg capsules extend lateral to the longitudinal excretory canals and pack the segment, leaving only the anterior portion free. Each capsule contains from 1 to 12 eggs. The eggs are spherical and 0.040 mm. in diameter.

Host.—Cat.

Dipylidium crassum, sp. nov.

The scolex is oval and tapers off as a blunt cone. Its length is 0.330 mm. and its width 0.380 mm. The four suckers are prominent. They are oval, 0.165 mm. by 0.145 mm. The rostellum is conical when retracted and more or less globular when extended. When retracted, it is 0.070 mm. long and 0.090 mm. wide at the base. It bears 5, occasionally 6 to 7 rows of rose-thorn-shaped hooks. The hooks in the anterior row are 0.010 mm. long; those in the posterior row 0.004 mm. long. The number of hooks varies from 15 to 20 in each row. The neck is moderately long and almost as broad as the scolex. It is either smooth or faintly scalloped. The first segments are much wider than long. They soon become square and then much longer than wide. The genital primordia appear between the 15th and the 20th segment. The mature and ripe segments are nearly rectangular. The posterior end of each segment is only slightly broader than the anterior end of the following segment and does not form a collar about it. The mature segments are 4 to 5 mm. long and 1 mm. wide. The ripe segments are 10 mm. long and 1.5 mm. to 2 mm. wide. The genital pores are prominent and a little posterior to the middle of the segment. Both the transverse and longitudinal excretory canals are distinct. The entire strobila attains a maximum length of 30 cm. and a maximum width of 2 mm.

The pyriform cirrus pouch extends medially and anteriorly from the genital pore to the longitudinal excretory canals. It measures 0.255 mm. long by 0.065 mm. wide. Medial to the cirrus pouch lie the coils of the vas deferens. The globular testes, 150 to 170 in number, fill most of the available space medial to the longitudinal excretory canals. They are 0.060 mm. in diameter.

The vagina opens posterior to the cirrus pouch. It sinuates almost directly medially to the longitudinal excretory canals and then curves irregularly posteriorly between the ovarian lobes. The ovaries are bilobed and very coarsely lobulated. The lateral ovarian lobe is slightly smaller than the median. Each ovary is oval, 0.545 mm. long and 0.255 mm. wide. A short distance posterior to each ovary lies an oval or reniform vitellarium, 0.200 mm. by 0.180 mm. The vitellarium is also very coarsely lobulated. It is as large as or occasionally even larger than the lateral ovarian lobe. Between the lobes of the ovary is a very narrow elongated seminal receptacle. The shell gland is small and compact. It lies close against the anterior aspect of the vitellarium. The uterus is usually distinctly visible in the postmature segments as a fine tubular network. The egg capsules extend lateral to the longitudinal excretory canals and, except in the anterior portion, almost completely fill the segment. Each egg capsule contains from 6 to 15 spherical eggs. Each egg is 0.035 mm. in diameter.

Host.—Dog.

Following is a descriptive table of all the known *Dipylidium:*

	D. caninum	D. echinorhyncoides
Total length	15 to 50 cm.	7 cm.
Maximum width	2 to 3 mm.	
Scolex width	0.327 mm.	scolex rhomboidal
Scolex length	0.365 mm.	
Suckers	large, oval, 0.165 by 0.150 mm.	elliptical
Rostellum	0.060 mm. long 0.100 mm. wide at base	cylindrical, base armed
Rows of hooks	4	16
Neck	long, thin, unsegmented	
Number of testes	110 to 175	
Ovary	bilobed, compact, 0.430 mm. by 0.275 mm.	
Vitellarium	compact, smaller than lateral ovarian lobe, 0.160 mm by 0.165 mm.	
Cirrus pouch	0.170 mm by 0.060 mm.	
Eggs per capsule	4 to 20	
Diameter of eggs	0.050 mm.	
Excretory canals	narrow	
Host	dog and cat	Megalotis cerdo
Locality	cosmopolitan	Egypt
Author	(Linnaeus); 1758	(Sonsino); 1889

	D. trinchesi	D. pasqualei
Total length	12 to 75 mm.	20 to 30 cm.
Maximum width	0.70 mm.	1 mm. to 2 mm.
Scolex width	0.212 mm. to 0.260 mm.	0.8 to 0.9 mm.
Scolex length		
Suckers	orbicular, prominent, 0.120 mm. in diameter	
Rostellum	0.100 mm. to 0.120 mm. wide	cylindrical
Rows of hooks	4	16
Neck	short, 0.8 mm. long	2 mm. long, unsegmented
Number of testes	25 to 32	few, about 50?
Ovary	bilobed, spherical, 0.050 mm. in diameter	bilobed
Vitellarium	spherical, 0.041 mm. in diameter	spherical
Cirrus pouch	cylindrical	cylindrical
Eggs per capsule	1	1
Diameter of eggs	0.046 mm. to 0.052 mm.	
Excretory canals	narrow	wide and prominent
Host	cat	cat
Locality	Italy; Egypt	Egypt
Author	Diamare; 1892	Diamare; 1893

	D. gervaisi	D. chyzeri
Total length	1 to 4 cm.	12 to 20 cm.
Maximum width	1 mm.	1.4 mm. to 1.6 mm.
Scolex width	0.250 mm.	0.352 mm. to 0.432 mm.
Scolex length	not sharply defined from neck. Rostl. to inf. limit suckers 0.15 mm.	
Suckers	prominent	
Rostellum	small	0.112 mm. long
Rows of hooks	8 to 12	13 to 14
Neck		
Number of testes		45 to 50
Ovary	bilobed	bilobed
Vitellarium	bilobed	smaller than ovarian lobe
Cirrus pouch		0.250 mm. long 0.120 mm. wide
Eggs per capsule	1	1
Diameter of eggs		0.052 mm.
Excretory canals	large	prominent
Host	Genette tigrina	cat
Locality	Eritrea	Budapest, Hungary
Author	Setti; 1895	von Ratz; 1897

	D. triseriale	D. monoöphoron
Total length	25 mm.	10 mm.
Maximum width	1 mm.	
Scolex width	0.300 mm.	0.150 mm.
Scolex length		
Suckers	0.100 mm. in diameter	0.070 mm. in diameter
Rostellum	0.150 mm. in diameter	0 070 mm. to 0.100 mm. in diameter
Rows of hooks	3	3
Neck		
Number of testes		
Ovary	bilobed	bilobed
Vitellarium	small, spherical	spherical
Cirrus pouch	cylindrical	cylindrical
Eggs per capsule	1	1
Diameter of eggs ...		
Excretory canals		
Host	Viverra civette	Viverra civette
Locality	Tunis; India	Tunis
Author	Lühe; 1898	Lühe; 1898

	D. oerleyi	D. sexcoronatum
Total length	5 to 11 cm.	10 to 30 cm.
Maximum width	2 mm.	2 mm.
Scolex width	0.390 mm.	0.350 mm. (0.340–0.380)
Scolex length	0.330 mm.	0.365 mm. (0.320–0.380)
Suckers	oval, not prominent	0.185 mm. by 0.160 mm.
Rostellum	conical, 0.067 mm. long, 0.071 mm. wide at base	0.065 mm. long 0.115 mm. wide
Rows of hooks	5	6, occasionally 7
Neck	short, thick	wide, short, scalloped
Number of testes	90 to 100	150 to 175
Ovary	bilobed	0.220 mm. by 0.295 mm. bilobed, compact
Vitellarium	smaller than ovarian lobe	compact, equal to lateral ovarian lobe, 0.125 mm. by 0.180 mm.
Cirrus pouch	curved, pyriform	0.290 mm. by 0.060 mm.
Eggs per capsule	more than 1	2 to 15
Diameter of eggs	0.025 mm.	0.025 mm.
Excretory canals	prominent	narrow but prominent
Host	cat	dog, cat
Locality	Budapest, Hungary	Hungary; England; India; United States
Author	von Ratz; 1900	von Ratz; 1900

	D. avicola	D. columbae
Total length	15 cm.	some centimeters
Maximum width	2 mm.	
Scolex width	0.480 mm.	0.800 mm.
Scolex length		
Suckers	0.140 mm. in diamater	
Rostellum	conical	conical
Rows of hooks	1 row? of 32 hooks	only 4 hooks (4 rows?)
Neck	not distinct	
Number of testes	numerous	
Ovary	bilobed, compact, 0.240 mm. in diameter	
Vitellarium	0.130 mm. in diameter	
Cirrus pouch	0.230 mm. to 0.300 mm. long	
Eggs per capsule	1	
Diameter of eggs	0.032 mm.	0.030 mm.
Excretory canals	prominent	
Host	Gyps kolbi	Columba sp.
Locality	South Africa	Sudan
Author	Fuhrmann; 1906	Fuhrmann; 1909

	D. zschokkei	D. dongolense
Total length	12 cm.	5 to 6 cm.
Maximum width	0.75 mm.	1 mm.
Scolex width	0.480 mm.	scolex rhomboidal
Scolex length		
Suckers	round, 0.150 mm. diam.	"not very large"
Rostellum	0 200 mm. wide	
Rows of hooks	3	4
Neck		short, wider than scolex
Number of testes	30	occupy all available space
Ovary	bilobed	spherical
Vitellarium	spherical	
Cirrus pouch	pyriform	
Eggs per capsule	1	1
Diameter of eggs		
Excretory canals		
Host	Cynictis penicillata	Genetta dongolana
Locality	Sekuma, South Africa	Africa or England (imported animal)
Author	Hungerbuhler; 1910	Beddard; 1913

	D. quinquecoronatum	D. walkeri
Total length	32 to 65 mm.	10 to 28 cm.
Maximum width	0 75 mm.	thin
Scolex width	0.370 mm. to 0.430 mm.	0 200 mm. to 0 360 mm.
Scolex length		
Suckers	0.130 mm. to 0.140 mm. in diameter	0 118 to 0 150 mm. in diameter
Rostellum	0.175 mm. to 0 215 mm. wide	elongate, 0.063 mm. to 0 100 mm. wide at base
Rows of hooks	5	6 to 7
Neck	0.260 mm. to 0.420 mm. long	short
Number of testes	46 to 58	225
Ovary	bilobed, 0.52 mm. to 0.065 mm. in diameter	bilobed, very diffuse
Vitellarium	small, 0.052 mm. in diameter	larger than lateral ovarian lobe
Cirrus pouch	bottle-shaped	0.050 mm. by 0.020 mm.
Eggs per capsule	1	1 to 15
Diameter of eggs	0.034 mm. to 0.038 mm.	0.035 mm.
Excretory canals		
Host ...	cat	dog
Locality	Granada, Spain	India
Author ..	Rodriquez Lopez-Neyra and Munoz Medina; 1921	Sondhi; 1923

	D. sp. of Kofend	D. gracile, sp. nov.
Total length	1.5 to 6 cm.	4 to 20 cm.
Maximum width	1 m.	2 mm.
Scoles width	0.276 mm.	0.460 mm. (0.420–0.500)
Scolex length		0.410 mm. (0.360–0.480)
Suckers		0.170 mm. by 0.172 mm.
Rostellum	conical	conical, 0.070 mm. long, 0.120 mm. wide at base
Rows of hooks	16 to 18	5 to 7
Neck	0.6 mm. long	long, unsegmented
Number of testes		200 to 250
Ovary	bilobed	bilobed, very diffuse, 0.290 mm. by 0.29 mm.
Vitellarium	spherical	diffuse, as large as lateral ovarian lobe, 0.165 mm. by 0.220 mm.
Cirrus pouch	0.105 mm. to 0.150 mm. long	pyriform, 0.255 mm. long, 0.075 mm. wide
Eggs per capsule		3 to 10
Diameter of eggs		0.030 mm.
Excretory canals		narrow, indistinct
Host	Felis capensis phillipsi	dog, cat
Locality	Sennaar (Sudan)	Oakland, California
Author	Kofend; 1921	Millzner; 1925

	D. compactum, sp. nov.	D. diffusum, sp. nov.
Total length	5 to 15 cm.	7 to 15 cm.
Maximum width	2 mm.	2.5 mm.
Scolex width	0.420 mm. (0.400–0.445)	0.460 mm. (0.450–0.470 mm.)
Scolex length	0.410 mm. (0.370–0.450 mm.)	0.450 mm. (0.420–0.500 mm.)
Suckers	oval, prominent, 0.175 mm. by 0.180 mm.	oval, slightly prominent, 0.175 mm. by 0.183 mm.
Rostellum	conical, 0.065 mm. long 0.100 mm. wide at base	conical, 0.075 mm. long, 0.110 mm. wide at base
Rows of hooks	6	6, occasionally 7 to 8
Neck	short, segmented	very short; scalloped
Number of testes	140 to 200	160 to 250
Ovary	bilobed, compact, 0.275 mm. by 0.280 mm.	bilobed, diffuse, 0.320 mm. by 0.315 mm.
Vitellarium	compact, equal to lateral ovarian lobe, 0.170 mm. by 0.180 mm.	compact, equal to lateral ovarian lobe, 0.130 mm. by 0.230 mm.
Cirrus pouch	pyriform, 0.245 mm. long, 0.060 mm. wide	pyriform, 0.255 mm. long, 0.070 mm. wide
Eggs per capsule	2 to 10	3 to 10
Diameter of eggs	0.036 mm.	0.033 mm.
Excretory canals	rather prominent	narrow
Host	cat	cat
Locality	Oakland, California	Oakland, California
Author	Millzner; 1925	Millzner; 1925

	D. longulum, sp. nov.	D. crassum, sp. nov.
Total length	5 to 12 cm.	10 to 30 cm.
Maximum width	2.3 mm.	2 mm.
Scolex width	0.440 mm. (0.400–0.460 mm.)	0.380 mm. (0.350–0.420 mm.)
Scolex length	0.410 mm. (0.380–0.440 mm.)	0.330 mm. (0.300–0.410 mm.)
Suckers	0.170 mm. by 0.175 mm.	0.165 mm. by 0.145 mm.
Rostellum	0.065 mm. long 0.110 mm. wide at base	0.095 mm. long 0.090 mm. wide at base
Rows of hooks	5	5, occasionally 6 to 7
Neck	long, unsegmented	wide and short
Number of testes	180 to 240	150 to 175
Ovary	bilobed, compact, 0.365 mm. by 0.400 mm.	very compact, bilobed, elongated, 0 545 mm. by 0.255 mm.
Vitellarium	compact, smaller than lateral ovarian lobe, 0.160 mm. by 0.240 mm.	compact, as large as lateral ovarian lobe, 0.290 mm. by 0.180 mm.
Cirrus pouch	pyriform, 0.275 mm. long, 0.075 mm. wide	pyriform, 0.255 mm. long, 0.065 mm. wide
Eggs per capsule	1 to 12	6 to 15
Diameter of eggs	0 040 mm.	0 035 mm.
Excretory canals	narrow	moderately prominent
Host	cat	dog
Locality	Oakland, California	Oakland, California
Author	Millzner; 1925	Millzner; 1925

KEY TO ALL KNOWN DIPYLIDIUM

- A Rostellum with over 8 rows of hooks
  - B Rostellum base unarmed
    - C Strobila 1–4 cm., long; scolex 0.250 mm. wide; rostellum bearing 8–12 rows of hooks *D. gervaisi*
    - CC Strobila 1.5–6 cm. long; scolex 0.276 mm. wide; rostellum bearing 16–18 rows of hooks *D. sp.* of Kofend
    - CCC Strobila 20–30 cm. long; scolex 0.800–0.900 mm. wide; rostellum bearing 16 rows of hooks *D. pasqualei*
    - CCCC Strobila 12–20 cm. long; scolex 0.350–0.450 mm. wide; rostellum bearing 13–14 rows of hooks *D. chyzeri*
  - BB Rostellum base armed
    - C Strobila 7 cm. long; rostellum bearing 16 rows of hooks *D. echinorhyncoides*
- AA Rostellum with 5 to 8 rows of hooks
  - B Only 1 egg in each egg capsule
    - C Strobila 6 cm. long; scolex 0.370–0.430 mm. wide; rostellum bearing 5 rows of hooks *D. quinquecoronatum*
  - BB More than 1 egg in each egg capsule
    - C Ovary compact
      - D Rostellum with 5 rows of hooks

E Strobila 5–11 cm. long; scolex 0.390 mm. wide; short thick neck; 90–100 testes . *D. oerleyi*

EE Strobila 10–30 cm. long; scolex 0.380 mm. wide; short thick neck; 150–175 testes . . *D. crassum*, sp. nov.

EEE Strobila 5–12 cm. long; scolex 0.440 mm. wide; long unsegmented neck; 180–240 testes *D. longulum*, sp. nov.

DD Rostellum with 6 rows of hooks

E Strobila 15–30 cm. long; scolex 0.350 mm. wide. *D. sexcoronatum*

EE Strobila 5–15 cm. long; scolex 0.420 mm. wide *D. compactum*, sp. nov.

CC Ovary diffuse

D Neck short, broad and scalloped

E Strobila 7–15 cm. long; scolex 0.460 mm. wide; rostellum bearing 6 rows of hooks *D. diffusum*, sp. nov.

DD Neck unsegmented and narrow

E Strobila 10–28 cm. long; scolex 0.260–0.360 mm. wide; 6–7 rows of hooks *D. walkeri*

EE Strobila 4–20 cm. long; scolex 0.460 mm. wide; 5–7 rows of hooks *D. gracile*, sp. nov.

AAA Less than 5 rows of hooks

B Adult forms in birds

C Rostellum bearing 1? row of about 32 hooks; strobila 15 cm long; scolex 0.480 mm. wide *D. avicola*

CC Rostellum bearing only 4 hooks (misprint for 4 rows?); strobila "some centimeters long"; scolex 0.800 mm wide *D. columbae*

BB Adult forms in mammals

C Rostellum with 3 rows of hooks

D Strobila 1 cm. long; scolex 0.150 mm. wide *D. monoophoron*

DD Strobila 2.5 cm. long; scolex 0.300 mm. wide *D. triseriale*

DDD Strobila 12 cm. long; scolex 0 480 mm. wide *D. zschokkei*

CC Rostellum with 4 rows of hooks

D Only 1 egg in each egg capsule

E Strobila 0.5–0.6 cm. long *D. dongolense*

EE Strobila 1.7–5 cm. long; scolex 0.212–0.260 mm. wide *D. trinchesi*

DD More than 1 egg in each capsule

E Strobila 15–40 cm. long; scolex 0.327 mm. wide; 4 to 20 eggs in each egg capsule *D. caninum*

COMPARISONS

The five new species described in this paper have from 5 to 7 rows of hooks. This at once separates them from the group composed of *D. gervaisi*, *D. sp.* of Kofend, *D. pasqualei*, *D. chyzeri*, and *D. echinorhyncoides*, all of which have over 8 rows and most of which have over 12 rows of hooks. Also, all of these, except *D. pasqualei* and *D. chyzeri*, are much smaller. The scolex of *D. pasqualei* is nearly twice the size of the scolex of any of the new species. Moreover, the five new species have more than one egg in each egg capsule, while both *D. pasqualei* and *D. chyzeri* have only one egg in each egg capsule. The number of rows of hooks also separates the five new species from the group composed of *D. avicola*, *D. columbae*, *D. monoöphoron*, *D. triseriale*, *D. zschokkei*, *D. dongolense*, *D. trinchesi* and *D. caninum*, all of which have less than 5 rows of hooks. *D. monoophoron*, *D. triseriale*, *D. trinchesi*, and *D. dongolense* are very much smaller worms. *D. avicola*, *D. triseriale*, *D. zschokkei*, *D. dongolense*, and *D. trinchesi* have only one egg in each egg capsule. Each of the five new species has more than one. The scolex of *D. columbae* is about twice the size, that of *D. monoöphoron* about one-third the size of the scolex of any one of the new species. *D. caninum* further differs from the new species in being a much larger worm, in the suckers being much larger in proportion to the rest of the scolex, in the neck being thinner and longer, in the mature and ripe segments being larger, and in the greater number of eggs in each brood pouch.

Although *D. quinquecoronatum* resembles three of the new species, *D. longulum*, *D. gracile*, and *D. crassum*, in having 5 rows of hooks, it is a smaller worm. Its suckers are smaller. Its rostellum is larger. It has only one-fourth as many testes in each segment. Its genital pores are nearer the anterior end of the segments. It has only one egg in each egg capsule.

The remaining *Dipylidium*, including the five new species, all have from 5 to 7 rows of hooks. They may be divided into two groups. The one, composed of *D. oerleyi*, *D. sexcoronatum*, *D. crassum* sp. nov., *D. longulum* sp. nov., and *D. compactum* sp. nov. has compact, very coarsely lobulated ovaries and vitellaria; the other, composed of *D. walkeri*, *D. diffusum* sp. nov., and *D. gracile* sp. nov. has diffuse very finely-lobulated ovaries and vitellaria.

D. crassum sp. nov., resembles *D. sexcoronatum* more closely than it does any other species. The strobila length is about the same in both, but the segments of *D. crassum* sp. nov. are much longer than those of *D. sexcoronatum*. *D. crassum* sp. nov. also lacks the scalloped appearance that is so characteristic of *D. sexcoronatum*. It has only 5, occasionally 6 or 7, rows of hooks, while *D. sexcoronatum* has 6, occasionally 7 rows. The most striking differences are in the mature segments. The ovaries and vitellaria of *D. crassum* sp. nov. are extremely elongated, both being nearly twice as long as they are wide. The ovaries and vitellaria of *D. sexcoronatum*, on the other hand, are nearly globular. The scolex of *D. crassum* sp. nov. is smaller than the scolex of any of the other new species. It has a short thick neck, thus differing from *D. longulum* sp. nov. and *D. gracile* sp. nov. It differs from all the other new species as well as from *D. walkeri* and *D. oerleyi*, in the shape of its ovaries and vitellaria which are elongated instead of globular. It differs from *D. walkeri* in having compact instead of very diffuse ovaries, in having a much larger scolex, and in having 5 instead of 6 to 7 rows of hooks. It differs from *D. oerleyi* in the strobila averaging a length three times as great, in having 150 to 175 testes in each proglottid instead of 90 to 100, and in the shape of its ovaries and vitellaria.

D. longulum sp. nov. does not closely resemble any of the *Dipylidium* previously known. It somewhat resembles one of the other new species, *D. compactum*, although it differs from it in many details. The scolex of *D. longulum* sp. nov. is very similar to that of *D. compactum* sp. nov. but it is somewhat wider and bears 5 instead of 6 rows of hooks. The neck is long and unsegmented, while that of *D. compactum* sp. nov. is short and segmented. *D. longulum* sp. nov. has 180 to 240 testes in each segment; *D. compactum* sp. nov. has 140 to 200. The ovaries and vitellaria are considerably larger than those of *D. compactum* sp. nov. The cirrus pouch is also larger than that of *D. compactum* sp. nov. *D. longulum* sp. nov. differs from *D. sexcoronatum* in having a larger scolex, in the neck being long and unsegmented instead of short and scalloped, in the testes being more numerous, and in the larger size of the ovaries and vitellaria. It differs from *D. oerleyi* in the scolex being much larger in all respects, in the neck being long instead of short and thick, in the testes being twice as numerous, and in the larger size of the reproductive organs and eggs.

D. compactum sp. nov. occupies a position intermediate between *D. longulum* sp. nov. and *D. sexcoronatum.* The main differences between this species and *D. longulum* sp. nov. have already been mentioned, but may be briefly repeated. *D. compactum* sp. nov. has a smaller scolex, 6 instead of 5 rows of hooks, a short segmented instead of a long segmented neck, fewer testes, and smaller ovaries and vitellaria. *D. compactum* sp. nov. is a shorter worm and does not show the scalloping which is characteristic of *D. sexcoronatum.* It further differs from *D. sexcoronatum* in having a larger scolex, larger testes, more globular ovaries, larger and more globular vitellaria, and larger eggs.

D. diffusum sp. nov. resembles *D. sexcoronatum* more than it does any of the other *Dipylidium.* There are, however, many important differences. The scolex instead of being oval and about the same width as the neck, as in *D. sexcoronatum,* forms almost a cube which is larger in diameter than the neck. The neck, like that of *D. sexcoronatum,* is very short and is scalloped by incomplete segmentations. The testes are more numerous and are larger than those of *D. sexcoronatum.*

D. gracile sp. nov. somewhat resembles *D. walkeri.* Like *D. walkeri,* its most striking characteristic is the extreme diffuseness of its ovaries and vitellaria. It differs from *D. walkeri* in being a shorter worm, in having a scolex nearly twice as large, and in the vitellaria being relatively smaller when compared with the ovaries. It differs from all the other new species, except *D. diffusum* sp. nov., in having diffuse instead of compact ovaries and vitellaria. It differs from *D. diffusum* sp. nov. in the scolex being smaller and not cuboidal, in having more testes, and in the ovaries being smaller. It differs from *D. sexcoronatum, D. oerleyi, D. crassum* sp. nov. and *D. diffusum* sp. nov. in having a long narrow unsegmented neck instead of a short thick or short scalloped neck.

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SUMMARY

Out of the 1230 *Dipylidium* from 28 dogs and 30 cats, only 3 specimens of *D. caninum* were found, and all three came from a single dog. *D. caninum* is therefore not only the least common *Dipylidium* in dogs and cats near Oakland, California, but it is the scarcest. *D. sexcoronatum* was found in both dogs and cats. It included 0.2 per cent of the worms from the dogs and 3 per cent of the worms from the cats. The remaining worms belonged to five new species. One of these, *D. gracile* sp. nov., was the commonest *Dipylidium* in both dogs and cats of this vicinity. It included 74 per cent of the worms from the dogs and 40 per cent of the worms from the cats. *D. crassum* sp. nov. included 25 per cent of the worms from the dogs. *D. compactum* sp. nov. was common in cats and included 40 per cent of those from the cats. The other two species, *D. longulum* sp. nov. and *D. diffusum* sp. nov., were found in smaller numbers, 10 per cent and 7 per cent, respectively, and only in cats.

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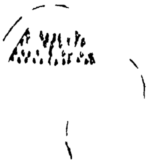
EXPLANATION OF PLATES

PLATE 33

Dipylidium caninum

Fig. 1. Rostellum and hooks. × 108.
Fig. 2. Front and lateral view of hook from the anterior row. × 860.
Fig. 3. Scolex and anterior portion of the strobila. × 17.5.
Fig. 4. Mature segment. × 17.5.
Fig. 5. Ripe segment. × 17.5.



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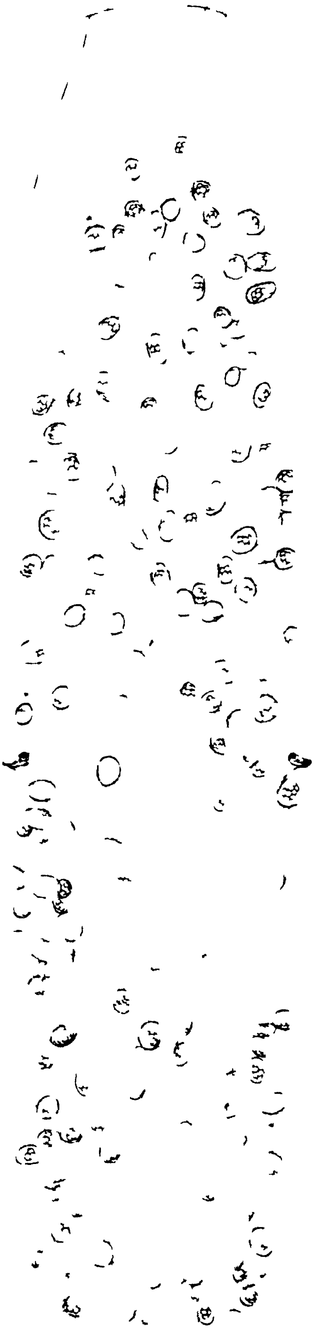

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PLATE 34

Dipylidium sexcoronatum

Fig. 6. Rostellum and hooks. × 108.
Fig. 7. Front and lateral view of hook from the anterior row. × 860.
Fig. 8. Scolex and anterior portion of the strobila. × 17.5.
Fig. 9. Mature segment. × 17.5.
Fig. 10. Ripe segment. × 17.5.

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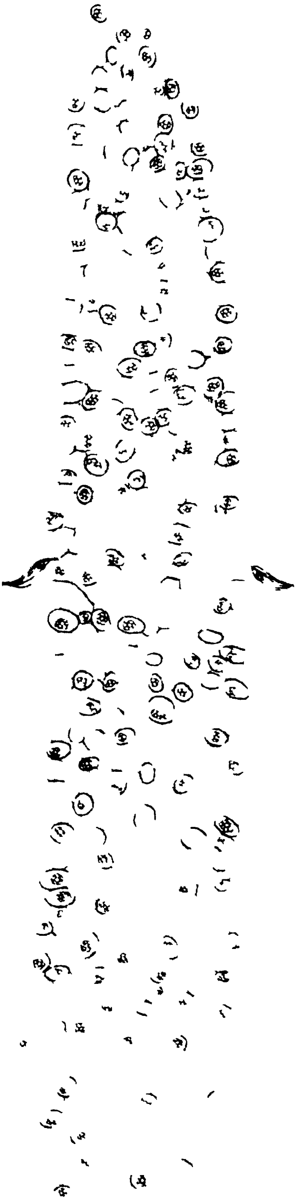

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PLATE 35

Dipylidium gracile, sp. nov.

Fig. 11. Rostellum and hooks. × 108.
Fig. 12. Front and lateral view of hook from the anterior row. × 860.
Fig. 13. Scolex and anterior portion of the strobila. × 17.5.
Fig. 14. Mature segment. × 17.5.
Fig. 15. Ripe segment. × 17.5.

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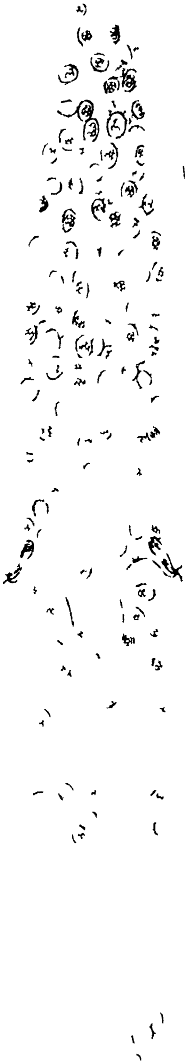

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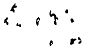
PLATE 36

Dipylidium compactum, sp. nov.

Fig. 16. Rostellum and hooks. × 108.
Fig. 17. Front and lateral view of hook from the anterior row. × 860.
Fig. 18. Scolex and anterior portion of the strobila. × 17.5.
Fig. 19. Mature segment. × 17.5.
Fig. 20. Ripe segment. × 17.5.



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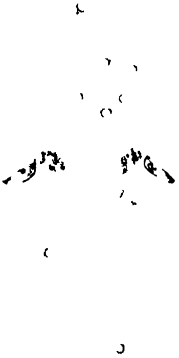

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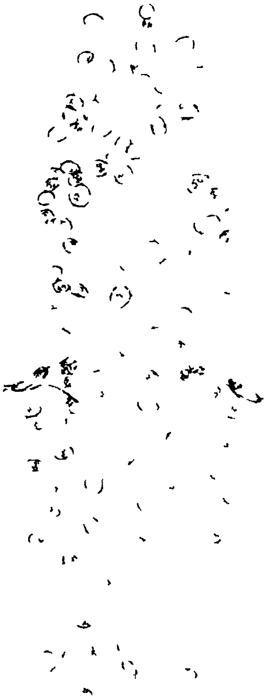

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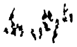
PLATE 37

Dipylidium diffusum, sp. nov.

Fig. 21. Rostellum and hooks. × 108.
Fig. 22. Front and lateral view of hook from the anterior row. × 860.
Fig. 23. Scolex and anterior portion of the strobila. × 17.5.
Fig. 24. Mature segment. × 17.5.
Fig. 25. Ripe segment. × 17.5.



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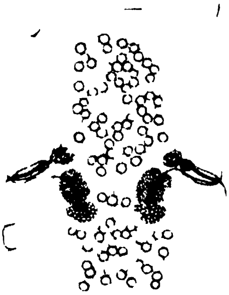

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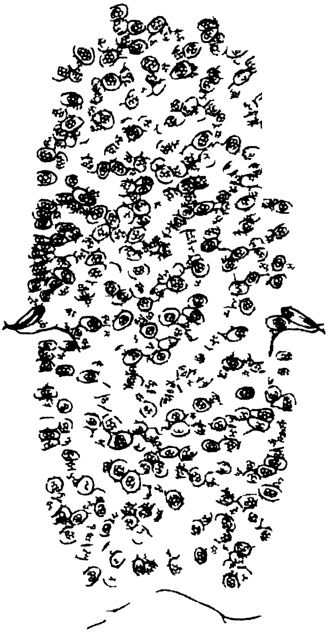

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PLATE 38

Dipylidium longulum, sp. nov.

Fig. 26. Rostellum and hooks. × 108.
Fig. 27. Front and anterior view of hook from the anterior row. × 860.
Fig. 28. Scolex and anterior portion of the strobila. × 17.5.
Fig. 29. Mature segment. × 17.5.
Fig. 30. Ripe segment. × 17.5.

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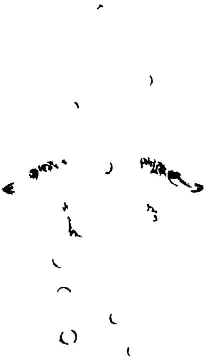

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PLATE 39

Dipylidium crassum, sp. nov.

Fig. 31. Rostellum and hooks. × 108.
Fig. 32. Front and anterior view of hook from the anterior row. × 860.
Fig. 33. Scolex and anterior portion of the strobila. × 17.5.
Fig. 34. Mature segment. × 17.5.
Fig. 35. Ripe segment. × 17.5.

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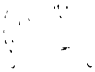

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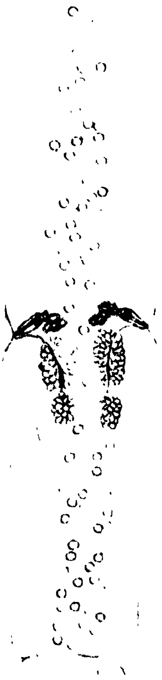

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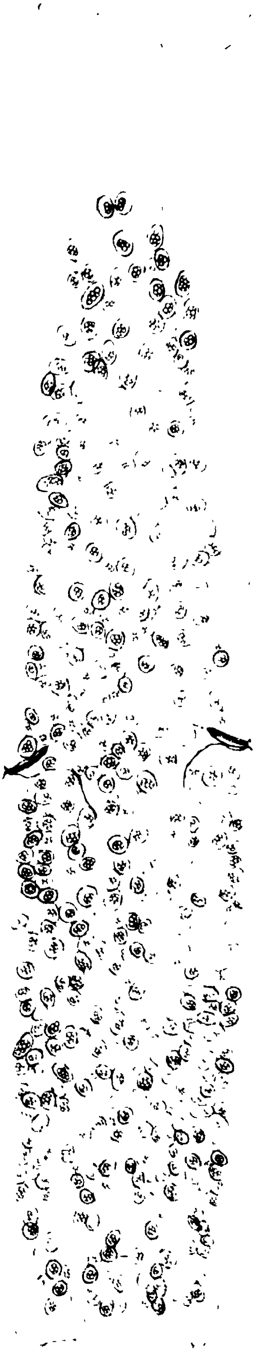

35

A USEFUL MODIFICATION OF A CLEARING FLUID FORMULATED BY SPALTEHOLZ

BY

FRANKLIN P. REAGAN

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A USEFUL MODIFICATION OF A CLEARING FLUID FORMULATED BY SPALTEHOLZ

BY

FRANKLIN P. REAGAN

A clearing method now familiarly known as the "Spalteholz method" has come widely into use in the study of developing bone and in the study of blood vessels which have been injected. This method, for these purposes, is generally regarded as superior to any other yet devised. Its clearing properties depend chiefly upon its content of oil of wintergreen (oil of *Gaultheria,* or methyl salicylate).

The Spalteholz method as employed by Sabin (1915) for the study of injected embryonic blood vessels is reviewed by Guyer (1921, p. 102). Sabin (1915) cleared in synthetic oil of wintergreen after the injected embryos had been fixed, and dehydrated. India ink or, in some cases, dilute silver nitrate constituted the injecting mass. Sabin found Carnoy's fluid to be the best fixing fluid, and especially desirable in that it removed tissue-pigments to such a degree that bleaching by means of hydrogen peroxide was found to be unnecessary as a procedure subsequent to fixing. These details may be profitably considered here for the reason that the modification of the Spalteholz method about to be described has been especially employed in the treatment of embryonic material for the study of blood vessels. A complete review of the Sabin technique will not be attempted.

The most objectionable feature of the Spalteholz method lies in the tendency of the clearing fluid, and even more, the tissue in it, to become yellow or brown after a short time. So disappointing has been the experience of many technicians who have used the method for the study of developing bone, that they have felt compelled to return to the old and objectionable potash-method of clearing that tissue.

If embryonic or foetal tissues are cleared first in benzol and then in pure benzyl benzoate they are seen to become most remarkably clear, particularly if they are illuminated by transmitted light.

Viewed in direct light they exhibit a bluish white or opalescent sheen which would seem to be a characteristic reaction of the tissue to benzyl benzoate. This appearance is also characteristic of the preparations of certain dealers whose representatives state that the clearing method employed is a carefully guarded secret; such preparations are of rare beauty except for the blue-white sheen.

Tissues cleared in several protracted changes of benzyl benzoate have little tendency to turn brown. This point of several changes is an important one. The more or less objectionable feature, the opalescent sheen, may be removed by the careful addition of a very small quantity of Natural Oil of Wintergreen. It is ordinarily possible to do this without danger of adding an amount of the latter oil sufficient to cause a later discoloration of the tissue, or of the fluid itself. In the matter of discoloration lies the danger of an excess of oil of wintergreen.

Such tendency toward maceration as may be detected when pure benzyl benzoate is used (especially if young embryos are to be cleared) may be overcome by the addition of a very small quantity of Beechwood Creosote to the mixture of benzyl benzoate and oil of wintergreen. Experience has shown that formalin-fixed embryos are not at all seriously macerated by this clearing treatment. This statement is based upon four years of experience with clearing mixtures composed largely of benzyl benzoate. Tissue fixed in Carnoy's fluid does frequently disintegrate in such clearing mixtures, especially if the tissue has been handled a great deal or dissected in the clearing mixture.

Directions for the clearing process may be briefly summarized as follows: After clearing by means of benzol, clear in pure benzyl benzoate; add just sufficient oil of wintergreen to remove any bluish or opalescent appearance in the tissue; then add a trace of beechwood creosote, giving the latter a total quantity of not more than 0.5 per cent. The natural oil is incomparably superior to the synthetic for this purpose.

The technique preceding the process of clearing may now be considered. The account of the treatment of tissue prior to clearing, as here presented, is mere statement of a personal opinion as to what seems to produce the best results. In the first place, undiluted India ink carefully filtered gives thoroughly satisfactory results. Then, formalin from 4 to 10 per cent plus a trace of acetic acid gives ideal fixation if the material is to be dissected later in water. The fixed tissue is somewhat clear, conveniently elastic, and easy to dissect or to section free-hand. Carnoy's fluid, on the other hand, shrinks the tissue, renders it tough and difficult to dissect, makes it opaque, and fails to remove tissue-pigments to such an extent that the preparation cannot be greatly improved by being bleached in hydrogen peroxide.

After being fixed in any standard formalin-acetic mixture for at least twenty-four hours the injected specimen should be washed for an hour in tap water. Following this it should be bleached in a mixture of three parts of water and one part of hydrogen peroxide; this is admittedly a strong bleaching fluid and is sure to produce a few bubbles in the tissue. These are likely to be equally numerous whether the bleaching fluid be strong or weak. Bubbles can generally be removed from the tissue without injury to the latter in so far as total preparations are concerned. Before the bleaching process has been carried very far the bubbles which form very conspicuously in the region of the mid-brain should be liberated through a puncture of the skin. The inconvenience of being obliged to remove a few bubbles is more than compensated for by the perfect transparency of the final preparation. Embryos may be bleached for periods of time varying between a few minutes and a few hours. Rather than depend upon set rules in matter of strength of bleaching fluid and time of exposure, one does well to make frequent observation of the tissue under treatment and make adjustments such as observation indicates. After the material has been bleached and the bubbles have been removed it should be washed a short time in water. It may then be returned to formalin provided it is not to be used for immediate study as a cleared object. When the material is dehydrated, however, all ordinary precautions should be exercised.

Little attempt has been made at a hard-and-fast formula or unvarying procedure. Rather it is thought best to outline a general procedure, the careful modification of which with reference to individual preparations will give the most satisfactory results.

The advantages of the method just outlined for clearing bulky objects to be studied later in their own clearing fluid may be summarized as follows:

1. Objects are rendered clearer than they can ordinarily be rendered by the Spalteholz method.

2. The superiority of the method suggested is best appreciated if the object must be studied in direct, rather than transmitted light.

3. Tissues so cleared have little tendency to turn objectionably brown, whereas Spalteholz preparations probably without exception become yellow or brown after a short time.

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THE EARLIEST BLOOD VESSELS OF THE MAMMALIAN EMBRYO STUDIED BY MEANS OF THE INJECTION METHOD

BY

FRANKLIN P. REAGAN

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THE EARLIEST BLOOD VESSELS OF THE MAMMALIAN EMBRYO STUDIED BY MEANS OF THE INJECTION METHOD

BY

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The youngest mammalian embryo hitherto injected for study is a pig of fourteen somites described by Sabin (1917). The youngest embryo of any kind studied by this method is a chick of nine somites, also described by Sabin. The pig embryo referred to must have had a circulation and, therefore, cardinal veins and a duct of Cuvier, if we may judge from the degree of development of its heart, its optic vesicle, and its brain-flexion. The venous structures mentioned are absent in the illustration of it. The injection of Sabin's youngest pig, which possessed fourteen somites, must therefore have been only partial. Sabin's youngest pig embryo in which injection approached completeness had twenty-three somites. It is not to be hoped that pig material younger than this will soon be studied, for the reason that the material presents difficulties peculiar to it, and very nearly unsurmountable. Complete injection need scarcely be hoped for if the rupture of the embryonic membranes or blood vessels is necessarily incident to injection.

In those rodents exhibiting the so-called 'inversion' of the germ layers and possessing a highly functional yolk sac which surrounds almost completely the embryo and its amnion, the omphalomesenteric vessels may be injected with relative ease while the heart is still beating. Beautiful and complete injections have been made of rat embryos having two and three somites.

Injected embryos are bleached, dehydrated, and finally cleared in a mixture of benzyl benzoate and (in small quantity) oil of wintergreen to which has been added a very slight trace of beechwood creosote described in the accompanying paper.

The accomplishment of injection of these early embryos makes possible for the first time a complete study of a vessel situated ventral to the alimentary tract and which early forms the entire proximal part, or artery of origin, of the omphalo-mesenteric artery. It has for some time been designated as the subintestinal artery, and has erroneously been regarded as an anastomosis between omphalomesenteric and umbilical arteries. Sabin regards it as a true omphalomesenteric vessel; the relationships of the vessel in the mouse have been known for a period of years to Professor Arthur Robinson, whose only published results (1923) are to be found in Cunningham's Text Book of Anatomy. In the figures of the text-book referred to, which deal with the subintestinal vessel, the latter is designated as a ventral aorta of the posterior region. The resolution of this vessel out of a yolk sac plexus along with intestinal concrescence has not been observed prior to the present study.

New information has been gained in the study of the primitive anterior cardinal vein (primary head vein plus transverse vein of the first interspace). Coincidentally with the backward migration of the heart the anterior cardinal vessel is lengthened by the addition to it of a (dorsal) portion of the primitive duct of Cuvier; vessels which originally entered the cephalic side of primitive duct of Cuvier maintain these relationships to it, but, inasmuch as the portion of the primitive duct which they enter becomes incorporated into the (definitive) anterior cardinal vein, they are later observed as tributaries of the anterior cardinal vein.

These vessels primitively entering the cephalic border of the duct of Cuvier have been figured but not mentioned by Streeter (1918, pl. 2, fig. 22) and by Heuser (1923, pl. 1, fig. 14). Probably the only attempt to interpret them has been that of Sabin, who designates them as veins of the somatopleure. They play an important part in the history of the mammary and facial veins. Their important early function is the superficial drainage of the branchial region.

The incorporation of the dorsal part of Cuvierian duct into anterior cardinal vein takes place by the establishment of new connections of the posterior cardinal vessel with the duct of Cuvier; that is to say, gradually the more infero-ventral portions of an originally indifferent and diffuse plexus lying between the upper posterior cardinal (common cardinal of Sabin) region and the duct of Cuvier take care of the blood from the posterior cardinal vein. With the progressive strengthening of these new connections the upper posterior cardinal (entire common cardinal of Sabin) degenerates. The following observations fail to support the conclusions of Sabin: The primitive duct of Cuvier is not a diffuse plexus lying lateral to the cardinal line for a distance of seven (Sabin, 1915) or less (Sabin, 1917) somites; only after the Cuvierian duct has formed as a direct connection between the transverse element of the primitive anterior cardinal vein and the potential venous end of the heart does this diffuse plexus between posterior cardinal vein and Cuvierian duct make its appearance; the Cuvierian duct is not a direct connection between dorsal aorta and potential venous end of the heart in all or even any of the interspaces nos. 2 to 7 inclusive. It is not even a connection of posterior or of 'common' cardinal with omphalomesenteric vein; anterior cardinal does not increase in length at the expense of the posterior cardinal vein (see Sabin, 1917, p. 80) but at the expense of the duct of Cuvier; the plexus inferior and dorsal to the duct of Cuvier (described by Sabin, 1917, p. 80, as a plexus "Continuous with the Cardinal System" and giving rise ultimately to the umbilical vein) contains the new connection of posterior cardinal with duct of Cuvier; that it does not give rise to the umbilical vein is indicated by the fact (compare Sabin's [1917] pl. 1, fig. 1) that the plexus is present still in embryos which in addition to it have a well established umbilical vein.

The posterior portion of the ventral aorta, instead of ending blindly, drains first into the dorsal-most region of the primitive duct of Cuvier, and then into the part of the latter which has become a part of the anterior cardinal vein.

In the mammal the posterior cardinal cannot be said to arise from diverticula off the dorsal aorta. From very early stages until the full number of somites is reached the neural plexus progresses more rapidly than does the posterior cardinal vein. It can be injected directly from the aortic intersegmentals in stages when corresponding levels of the posterior cardinal vessel cannot (consult also Sabin [1917], pl. 1, fig. 1, and pl. 4, fig. 3). This observation has been made in injected rat embryos with as few as seven somites.

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University of California.

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THE BIOLOGICAL RELATIONSHIPS OF LEISHMANIA AND CERTAIN HERPETOMONADS

BY

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THE BIOLOGICAL RELATIONSHIPS OF LEISHMANIA AND CERTAIN HERPETOMONADS

BY

EDNA HANNIBAL WAGENER AND DOROTHY ANN KOCH

CONTENTS

	PAGE
Introduction	365
Acknowledgments	366
Comparison of morphology	366
Immunological reactions with antiserums	368
Skin reactions in sensitized animals	373
Attempted experimental infection of mice	377
Summary and conclusions	379
Literature cited	380
Explanation of plates	382

INTRODUCTION

During the course of the work on skin reactions in rabbits immunized to two species of *Leishmania* (Wagener, 1923), the possibility of using biological methods to differentiate between *Leishmania* and herpetomonads from insects suggested itself. The difficulties of differentiating these two genera on morphology alone are realized by all workers in this field. In fact, their resemblances are so close that Fantham and Porter (1914) state that "it may be expected that the various Leishmanioses occurring in different parts of the world, will prove to be insect-born herpetomoniases." Laveran and Franchini (1913, 1914*a*, 1914*b*, 1919, 1920) as well as Fantham and Porter (1914, 1915*a*, 1915*b*, 1916) claim to have produced experimental herpetomoniases resembling leishmaniasis in mice and other animals from insect herpetomonads. These findings have not been verified by the more recent workers including Chatton (1919), Noller (1920), Glaser (1922), Becker (1923), or Yamasake (1924). Short (1923) has repeatedly failed to infect mice with *Herpetomonas ctenocephali*.

The more striking, therefore, is the work of Strong (1924), who has passed flagellates found infecting *Euphorbia* in Panama in turn through its insect host, *Chariesterus cuspidatus,* through the digestive tract of a lizard, *Cnemidophorus limnescatus gaigii,* and subcutaneously into a monkey. Sixteen days after inoculation an ulcerated lesion which showed a few *Leishmania*-like bodies developed in the monkey at the site of inoculation. We have undertaken to carry on cross-agglutination and skin reactions with five strains of *Leishmania* and with *H. ctenocephali.* We have also attempted to infect animals with flagellates from several other insects and to determine the life cycle of one of these flagellates (*H. ctenocephali*) on artificial media.

ACKNOWLEDGMENTS

We are indebted to Professor F. G. Novy of the University of Michigan for the cultures of *Leishmania tropica;* to Dr. E. E. Tyzzer of Harvard University for the cultures of *Leishmania infantum;* to Dr. C. Nicolle, of Tunis, Africa, for a strain of *Leishmania donovani* isolated in Tunis; to Dr. C. W. Young, of Peking Union Medical School, for a strain of *Leishmania donovani* isolated from an infantile case of Kala Azar; and to Dr. H. Noguchi, for the strain of *Leishmania brasiliensis.* We also wish to express our appreciation to Professor Charles A. Kofoid and Professor J. N. Force for many kindly suggestions made during the course of this work, and to Dr. Olive Swezy for the drawings on plate 43.

COMPARISON OF MORPHOLOGY

In comparing fresh preparations on slides stained with Wright's stain, the worker is struck with the close resemblance of the *Leishmania* parasites in culture to the insect herpetomonads. The differences become more pronounced in preparations which have been fixed in hot Schaudinn's fluid and carefully stained in iron haematoxylin. Yamasaki (1924), also, has considered these forms distinct.

Leishmania passes through a definite life cycle in its growth in artificial media. Two to three weeks are required to complete the cycle. The variations in time are due to differences in temperature and to the richness of the medium. Incubation at 22° C. or the presence of dextrose in the medium, hastens growth, but appreciably shortens the life of the culture. We have therefore omitted dextrose, and have incubated our cultures at room temperature (about 18° C.). In cultures from three to seven days old the prevailing type of *Leishmania* is the flagellate, present either singly or in rosettes (pl. 42, figs. 45 and 46). The greatest growth takes place from the seventh to the twenty-first day of incubation. The long slender flagellates shorten and thicken until the prevailing type becomes a round or slightly oval form with a long flagellum attached. Many dividing forms are found. The blepharoplast divides first (pl. 42, figs. 39 and 47). In the beginning only one flagellum is present but in later stages (pl. 42, fig. 50) a single flagellum is attached to each blepharoplast. These dividing forms may be found singly, or more frequently, in masses (pl. 42, fig. 42) or rosettes. The prevailing type in cultures of three to four weeks' incubation is again the slender flagellate with a very occasional, round, dividing form present. In still older cultures only a few very small spherical forms with flagella six or seven times the length of the body are present. None of these has ever been seen dividing (pl. 42, figs. 40 and 41). No organisms of any type have ever been found in cultures more than fifty days old.

To maintain the best cultures, transplants should be made every fourteen to twenty-one days or during the period of rapid multiplication. We have had successful transplants, however, from cultures forty-two days old, where only occasional, very small, round forms were present. Row (1922) has described round forms in cultures but he does not consider them the dividing stages. We have never observed these round forms developing the protecting membrane which he describes, or without a flagellum in preparations stained by haematoxylin. In preparations stained in Wright's stain the flagellum frequently is indistinct and a pseudo-membrane appears around the organism due to the drying of the film. Row does not state how his preparations were stained.

The insect herpetomonads complete this same life cycle but it appears to cover a much shorter period. At the end of five days' incubation at room temperature (18° C.) small round, dividing forms may be found in cultures of *Herpetomonas ctenocephali* (pl. 40, figs. 11, 12, 13, and 14). The flagellum in these stages is very short (half the length of the body) or is entirely lacking. After this stage is passed, long, slender flagellates develop, but the cultures die out after ten to twelve days incubation.

The best subcultures have always been obtained from five to seven day cultures. The prevailing type found in forty-eight hour cultures is a large granular flagellate with a short thick flagellum (pl. 40, figs. 6 and 7). The results of daily examinations of cultures of *H. ctenocephali* are shown in table 1.

TABLE 1

PREDOMINATING FORMS OF HERPETOMONAS CTENOCEPHALI IN STAINED SMEARS FROM CULTURES ON N.N.N. MEDIUM

Day	Figs.	Most abundant forms	Rarer forms
1	1, 2, 3	Rounded forms	
2	4, 5	Large flagellates	
3	6, 7	Flagellates (forms coarsely granular)	
4	8, 9	Small rounded forms, dividing	Small flagellates
5	10	Small finely granular flagellates (rare)	
6	11, 12 13, 14	Rounded forms, dividing and single Young rosettes (heaviest growth here)	Solitary, highly vacuolated flagellates
7	15, 16, 17	Small delicate flagellates	Small rounded forms dividing
8	18, 19	Very small flagellates	Large plasmodial-like bodies with nucleus, blepharoplast and parabasal very distinct
10	20, 21	Short, rounded flagellates with heavy nuclei	
11	22, 23 24, 25	Small flagellates with heavy flagellum	Large flagellates

IMMUNOLOGICAL REACTIONS WITH ANTISERUMS

Cultures of *Leishmania tropica, L. donovani* (Peking strain), *L. donovani* (Tunis strain), *L. brasiliensis*, *L. infantum,* and *Herpetomonas ctenocephali* were grown on Young and Van Sant's (1923) modification of N.N.N. medium of a pH 7.7. These cultures were allowed to grow at room temperature (19° C.) for twelve days. At the end of this time the growth was washed off in sterile saline and decanted into a centrifuge tube. The organisms were washed once in sterile saline, to rid them of any blood, and were collected in the bottom of the tube by centrifuging for five minutes. With the suspensions of living flagellates thus prepared, two rabbits were immunized to each of four strains of *Leishmania* and one strain of *Herpetomonas ctenocephali.* Each rabbit received intravenously 2 c.c. of the flagellates on the fifth, eighth, and thirteenth day of the month.

Fresh suspensions of flagellates were prepared for each inocluation. Five days after the last inoculation 20 c.c. of heart's blood was removed from each rabbit inoculated. The blood was placed in large test tubes in the ice box and allowed to clot. The following day the serum was removed for serological work.

Originally we planned to carry out cross-complement fixation tests as well as cross-agglutination tests on all sera and antigens. It was found impractical, however, to carry out the complement fixation reactions as all antigens prepared from cultures of *L. tropica* and *H. ctenocephali* were anticomplementary.

The technique used for the agglutination tests was as follows: Growth from twelve day cultures on N.N.N. medium was washed off with 0.9 per cent NaCl solution and the organisms collected by centrifuging. It was necessary to wash the flagellates twice to remove all traces of blood. The growth was then suspended in 0.9 per cent NaCl solution. On account of the difference in size of the flagellates of the various strains used, it was impossible to standardize these antigens by direct count or by use of the Hopkins tube. Sufficient 0.9 per cent sodium chloride was added to make the suspension of flagellates of such consistency that print was invisible when the 15 c.c. centrifuge tube containing the antigen was held in front of a printed page. A drop of toluol was added to each antigen before storing in the ice box.

Each immune serum as well as serum from a normal control animal was used in dilutions ranging from 1–10 to 1–600 as shown in table 2. Macroscopic tests were made, 1 c.c. of the dilution of serum being placed in each agglutination tube. To each tube 0.1 c.c. of antigen was added. This was sufficient to give a slightly opalescent appearance to the suspension. A saline control was run with each series.

The racks of tubes were then placed in a 56° water bath for two hours. At the end of this time the reactions were read and recorded and the tubes placed in the ice box to be read again the following morning. Only those tubes containing heavy flocculent clumps of flagellates were recorded as positive. There was no difficulty in distinguishing those tubes in which complete clearing occurred from those with the pseudo-agglutinations which often occur with flagellated organisms. In a pseudo-agglutination the clumps are very small and break up when the tube is gently tapped.

The results obtained were very striking (see table 2). The highest titers were always obtained with the homologous serum. Cross-

TABLE 2

TABLE SHOWING AGGLUTINATION REACTIONS OCCURRING BETWEEN THE LEISHMANIA AND A HERPETOMONAD AND THEIR HOMOLOGOUS AND HETEROLOGOUS ANTISERUMS

No. of rabbit	Species immunised to	No. of intravenous injections	Antigens					
			L. tropica	L. brasiliensis	L. donovani (Tunis strain)	L. donovani (Peking strain)	L. infantum	Herpetomonas ctenocephali
151	L. tropica	3	1-10++	1-10+++	1-10+++			1-10++
			1-20+++	1-20+++	1-20+++			1-20–
			1-40+++	1-40+++	1-40+++			1-40–
			1-80+++	1-80+++	1-80+++			1-80–
			1-100+++	1-100+++	1-100+++			1-100–
			1-200+++	1-200+	1-200+++			1-200–
			1-300+++	1-300+–	1-300+			1-300–
			1-400+++	1-400–	1-400+–			1-400–
			1-500+	1-500–	1-500–			1-500–
			Control–	Control–	Control–			Control–
152	L. tropica	3	1-10+++	1-10+++	1-10++	1-10++	1-10+++	1-10+
			1-20+++	1-20+++	1-20+++	1-20+++	1-20+++	1-20–
			1-40+++	1-40+++	1-40+++	1-40++	1-40+	1-40–
			1-80+++	1-80+++	1-80+++	1-80–	1-80–	1-80–
			1-100+++	1-100+++	1-100+++	1-100–	1-100–	1-100–
			1-200+++	1-200++	1-200++	1-200–	1-200–	1-200–
			1-300+++	1-300+	1-300+	1-300–	1-300–	1-300–
			1-400+++	1-400+–	1-400–	1-400–	1-400–	1-400–
			1-500+++	1-500–	1-500–	1-500–	1-500–	1-500–
			1-600+	Control–	Control–	Control–	Control–	Control–
			Control–					
153	L. donovani (Peking strain)	3	1-10++	1-10++	1-10+++	1-10++	1-10+++	1-10+–
			1-20+++	1-20+++	1-20+++	1-20+++	1-20+++	1-20–
			1-40+++	1-40+++	1-40++	1-40+++	1-40+++	1-40–
			1-80++	1-80+	1-80–	1-80+++	1-80+–	1-80–
			1-100+	1-100+–	1-100–	1-100++	1-100–	1-100–
			1-200–	1-200–	1-200–	1-200+–	1-200–	1-200–
			1-300–	1-300–	1-300–	1-300–	1-300–	1-300–
			1-400–	1-400–	1-400–	1-400–	1-400–	1-400–
			1-500–	1-500–	1-500–	1-500–	1-500–	1-500–
			Control–	Control–	Control–	Control–	Control–	Control–
154	L. donovani (Peking strain)	3	1-10++	1-10+++	1-10+++			1-10+
			1-20+++	1-20+++	1-20++			1-20–
			1-40+++	1-40+++	1-40+			1-40–
			1-80+–	1-80+	1-80–			1-80–
			1-100–	1-100–	1-100–			1-100–
			1-200–	1-200–	1-200–			1-200–
			1-300–	1-300–	1-300–			1-300–
			1-400–	1-400–	1-400–			1-400–
			1-500–	1-500–	1-500–			1-500–
			Control–	Control–	Control–			Control–
155	L. brasiliensis	3	1-10++	1-10++	1-10++	1-10++	1-10+++	1-10+–
			1-20+++	1-20+++	1-20+++	1-20+++	1-20+++	1-20–
			1-40+++	1-40+++	1-40+++	1-40+	1-40++	1-40–
			1-80+++	1-80+++	1-80+++	1-80–	1-80+	1-80–
			1-100+++	1-100+++	1-100++	1-100–	1-100+–	1-100–
			1-200+++	1-200+++	1-200+	1-200–	1-200–	1-200–
			1-300+++	1-300+++	1-300–	1-300–	1-300–	1-300–
			1-400++	1-400+	1-400–	1-400–	1-400–	1-400–
			1-500+	1-500–	1-500–	1-500–	1-500–	1-500–
			1-600–	1-600–	Control–	Control–	Control–	Control–
			Control–	Control–				

TABLE 2—(*Continued*)

No. of rabbit	Species immunized to	No. of intravenous injections	Antigens: L. tropica	L. brasiliensis	L. donovani (Tunis strain)	L donovani (Peking strain)	L. infantum	Herpetomonas ctenocephali
156	L. brasiliensis	3	1-10+++	1-10++	1-10++			1-10++
			1-20+++	1-20+++	1-20+++			1-20-
			1-40+++	1-40+++	1-40+++			1-40-
			1-80+++	1-80+++	1-80++			1-80-
			1-100+++	1-100+++	1-100+			1-100-
			1-200+++	1-200+++	1-200+-			1-200-
			1-300++	1-300+	1-300-			1-300-
			1-400+-	1-400+-	1-500-			1-500-
			Control-	1-500-	Control-			Control-
				Control-				
157	L infantum	3	1-10++	1-10++	1-10+++	1-10++	1-10++	1-10+
			1-20+++	1-20+++	1-20+++	1-20+++	1-20+++	1-20-
			1-40+++	1-40++	1-40+++	1-40+++	1-40+++	1-40-
			1-80++	1-80+	1-80+++	1-80+	1-80+++	1-80-
			1-100+	1-100+-	1-100+++	1-100-	1-100+++	1-100-
			1-200+-	1-200-	1-200+	1-200-	1-200++	1-200-
			1-300-	1-300-	1-300-	1-300-	1-300+	1-300-
			1-400-	1-400-	1-400-	1-400-	1-400+-	1-400-
			1-500-	1-500-	1-500-	1-500-	1-500-	1-500-
			Control-	Control-	Control-	Control-	Control-	Control-
158	L infantum	3	1-10+++	1-10++	1-10++			1-10+
			1-20+++	1-20+++	1-20+++			1-20-
			1-40+++	1-40+	1-40+++			1-40-
			1-80+++	1-80+-	1-80+++			1-80-
			1-100++	1-100-	1-100+++			1-100-
			1-200+	1-200-	1-200+			1-200-
			1-300-	1-300-	1-300+-			1-300-
			1-400-	1-400-	1-400-			1-400-
			1-500-	1-500-	1-500-			1-500-
			Control-	Control-	Control-			Control-
159	H. ctenocephali	3	1-10++	1-10+	1-10++	1-10+	1-10++	1-10+++
			1-20+	1-20-	1-20-	1-20-	1-20-	1-20+++
			1-40-	1-40-	1-40-	1-40-	1-40-	1-40+++
			1-80-	1-80-	1-80-	1-80-	1-80-	1-80+
			1-100-	1-100-	1-100-	1-100-	1-100-	1-100+-
			1-200-	1-200-	1-200-	1-200-	1-200-	1-200-
			1-300-	1-300-	1-300-	1-300-	1-300-	1-300-
			1-400-	1-400-	1-400-	1-400-	1-400-	1-400-
			1-500-	1-500-	1-500-	1-500-	1-500-	1-500-
			Control-	Control-	Control-	Control-	Control-	Control-
160	H. ctenocephali	3	1-10++	1-10+	1-10+			1-10++
			1-20+	1-20-	1-20-			1-20+++
			1-40-	1-40-	1-40-			1-40+++
			1-80-	1-80-	1-80-			1-80+++
			1-100-	1-100-	1-100-			1-100+
			1-200-	1-200-	1-200-			1-200-
			1-300-	1-300-	1-300-			1-300-
			1-400-	1-400-	1-400-			1-400-
			1-500-	1-500-	1-500-			1-500-
			Control-	Control-	Control-			Control-
	Normal control		1-10+	1-10++	1-10+	1-10+	1-10++	1-10++
			1-20+-	1-20-	1-20-	1-20-	1-20-	1-20-
			1-40-	1-40-	1-40-	1-40-	1-40-	1-40-
			1-80-	1-80-	1-80-	1-80-	1-80-	1-80-
			1-100-	1-100-	1-100-	1-100-	1-100-	1-100-
			1-200-	1-200-	1-200-	1-200-	1-200-	1-200-
			1-300-	1-300-	1-300-	1-300-	1-300-	1-300-
			1-400-	1-400-	1-400-	1-400-	1-400-	1-400-
			1-500-	1-500-	1-500-	1-500-	1-500-	1-500-
			Control-	Control-	Control-	Control-	Control-	Control-

agglutination occurred with the other strains but always at a lower titer. Partial agglutination occurred in normal serum at low dilutions. *Herpetomonas ctenocephali* was agglutinated only by its homologous serum. Sera from animals immunized to *Leishmania* were no more active than the normal serum control, while *Herpetomonas* antisera did not agglutinate any of the strains of *Leishmania.*

Shortly after we had completed this portion of our work, Noguchi (1924) published similar work done by him with two of our strains of *Leishmania.* Four strains, *L. tropica, L. brasiliensis* (presumably the same strain with which he supplied us), *L. donovani* (Peking), and *L. infantum* (our strains) were grown in semifluid *Leptospira* medium for thirty days at 18° C. At the end of this time antisera were added to the cultures. Noguchi states that

> The anti-*donovani* and anti-*infantum* serums agglutinated *L. donovani* and *L. infantum* reciprocally—that is, these two strains were serologically identical. On the other hand neither of these serums showed any agglutinating property for *Leishmania tropica* or *Leishmania brasiliensis.* The anti-*tropica* serum agglutinated only the *tropica* strain and the anti-*brasiliensis* only the *brasiliensis.* The titers of these anti-serums were such that a 1–10 dilution caused a prompt and powerful agglutination, while 1–100 was moderately active against the homologous strains.

As has been noted from table 2 the results obtained by us differ from Noguchi's. This is easily explained by the differences in technique employed; e.g., we used killed antigens of standard strength, while Noguchi used living cultures, which are always variable in amount of growth and reaction to anitsera. Nor does Noguchi state the time of incubation after adding the antisera, although "prompt" would imply an immediate reaction. We do not feel, therefore, that the results of either are invalidated, but that because of the technique used by us it is even more definitely established that *Herpetomonas ctenocephali* is not related to the *Leishmania,* if we accept Wells' (1925) view in regard to biological specificity.

It would have been interesting if Strong (1924) had used cultural and serological methods to compare the plant *Herpetomonad* with which he started his infection experiments, with that occurring in the lesion of the monkey.

SKIN REACTIONS IN SENSITIZED ANIMALS

The same rabbits from which the antisera had been taken were tested for skin reactions. Seven days after they had received their last intravenous inoculation, the rabbits were shaved on both sides.

For these experiments we used New Zealand red rabbits because of their white skins, but on shaving them it was found that five of the ten immunized rabbits were of mixed breed and had dark hair follicles. They were therefore excluded from the tests. The following day the remaining five rabbits and one normal control animal received 0.2 c.c. of alkaline extracts of each of the strains of *Leishmania* and of *Herpetomonas ctenocephali.* These extracts were prepared by the same technique that had been previously used (Wagener, 1923) for skin tests. The growth from twenty to thirty cultures was washed off in saline and packed by rapid centrifugalization for one half-hour. One part of the packed organisms was then diluted with four parts of Coca's (1922) extracting fluid. A few drops of toluol were added as a preservative and the whole was stored in the ice box for three days before using. Each rabbit was inoculated intradermally with the following antigens, *L. tropica*, *L. brasiliensis*, *L. donovani* (Tunis), *L. donovani* (Peking), *L. infantum*, *H. ctenocephali*, *Trypanosoma lewisi*, and a control of Coca's extracting fluid.

The reactions were read at the end of twenty-four and forty-eight hours and observed again after ninety-six hours. A red and indurated area of from 4 to 5 mm. in diameter which appeared at the end of twenty-four hours and reached its height at the end of forty-eight hours was considered positive. All of the rabbits which had been injected intravenously with living *Leishmania* reacted to all the *Leishmania* antigens, but not to the *Herpetomonas*, *Trypanosoma,* or control antigens. The normal control showed reactions to none of the antigens (see table 3). Thus rabbits injected with any of the strains of *Leishmania* become hypersensitive to all strains of *Leishmania* but not to the unrelated protein of *Herpetomonas* or *Trypanosoma.*

In order to check our results on rabbits, two white guinea pigs were each given 5 c.c. of a thick suspension of twenty twenty-one-day cultures of *L. tropica.* Skin tests were made using *L. tropica* extract eight and fifteen days after the sensitizing inoculation. Both animals gave positive skin tests each time they were tested. A normal control guinea pig (see table 4) gave no skin reaction.

TABLE 3

SKIN REACTIONS OCCURRING ON SENSITIZED RABBITS AT THE END OF 48 HOURS

No. of rabbit	Species immunized to	Reaction on skin of rabbit	Antigens used for intradermal injections							
			L. tropica	L. brasiliensis	L. donovani (Tunis strain)	L. donovani (Peking strain)	L. infantum	Herpetomonas ctenocephali	Trypanosoma lewisi	Control antigen
151	L. tropica	Necrosis	3 mm.							
		Induration	5 mm.	5 mm.	6 mm.	3 mm.	5 mm.	—	—	—
		Redness	10 mm.	5 mm.	7 mm.	4 mm.	5 mm.	—	—	—
152	L. tropica	Necrosis	3 mm.							
		Induration	11 mm.	5 mm.	13 mm.	4 mm.	3 mm.	—	—	—
		Redness	11 mm.	5 mm.	13 mm.	4 mm.	5 mm.	—	—	—
153	L. donovani (Peking strain)	Induration	12 mm.	5 mm.	7 mm.	4 mm.	4 mm.	—	—	—
		Redness	14 mm.	5 mm.	7 mm.	5 mm.	5 mm.	—	—	—
155	L. brasiliensis	Induration	19 mm.	4 mm.	5 mm.	3 mm.	5 mm.	—	—	—
		Redness	19 mm.	6 mm.	5 mm.	4 mm.	5 mm.	—	—	—
158	L. infantum	Induration	12 mm.	4 mm.	8 mm.	4 mm.	6 mm.	—	—	—
		Redness	12 mm.	6 mm.	8 mm.	5 mm.	6 mm.	—	—	—
	Normal control	Induration	—	—	—	—	—	—	—	—
		Redness	5 mm.	—	—	—	—	—	—	—

Similar tests were made on a guinea pig sensitized with living *Herpetomonas ctenocephali* from ten day cultures and later inoculated intradermally with the same alkaline extract of *H. ctenocephali* which was used for intradermal tests on the rabbits. The guinea pig gave a positive skin test to *H. ctenocephali* antigen but not to the control antigen. A normal control pig gave no reaction (see table 5).

Thus both rabbits and guinea pigs may be sensitized with living cultures of protozoans as shown by the intradermal reaction, in a manner similar to their sensitization with bacteria or fungi.

TABLE 4

RESULTS OF SKIN REACTIONS ON GUINEA PIGS SENSITIZED WITH LEISHMANIA TROPICA

Number of guinea pig	Amount of living culture given	Date of inoculation	Method of injection	Date of first intradermal test	Results after 48 hours	Date of second intradermal test	Results after 48 hours	Control antigen
167	5 c.c.	6/21/24	Intraperitoneal	6/29/24	Necrosis 2.5 mm Induration 7 mm. Redness 10 mm.	7/6/24	Necrosis 3 mm. Induration 6 mm. Redness 6 mm.	Negative
168	5 c.c.	6/21/24	Intraperitoneal	6/29/24	Necrosis 3 mm Induration 5 mm. Redness 8 mm	7/6/24	Necrosis 2 mm. Induration 5 mm. Redness 6 mm.	Negative
Normal control	—	—	—	6/29/24	Negative	—	—	Negative
Normal control	—	—	—	—	—	7/6/24	Negative	Negative

TABLE 5

Results of Inoculation of Animals with Insect Herpetomonads

No. of mouse	Material	Injection			Blood examination		Intradermal injection			Autopsy findings	
		Method	Amount	Date	Date	Result	Date	Reaction	Control	Results of smears	Results of culture
1	Saline suspension of gut of box elder bug.	Intraperitoneal	1 c c	May 19	May 23	Neg	June 16	+? Red, indurated 8 x 2	Neg.	Neg.	
2	Saline suspension of gut of box elder bug.	Intraperitoneal	1 c c	May 19	May 23	Neg				Neg.	Neg.
3	Saline suspension of gut of box elder bug.	Intraperitoneal	1 c c	May 19	May 23	Neg.	June 16	Neg.	Neg	Neg.	
1A	Saline suspension of 10 16-day cultures of H. ctenocephali	Intraperitoneal	¾ c c	June 2	June 3 June 7	Neg				Neg.	Neg.
2A	Saline suspension of 10 16-day cultures of H. ctenocephali.	Intraperitoneal	¾ c c	June 2	June 3 June 7	Neg				Neg.	Neg.
3A	Saline suspension of 10 16-day cultures of H. ctenocephali	Intraperitoneal	¾ c c	June 2	June 3 June 7		June 16	Slight induration	Neg.	Emaciated Neg.	
4A	Saline suspension of 10 16-day cultures of H. ctenocephali	Intraperitoneal	1 c c	June 2	June 3 June 7	Neg.	June 16	Neg	Neg		
5A	Saline suspension of 10 16-day cultures of H. ctenocephali.	Intravenous	½ c c.	June 2	June 3 June 7	Neg	June 16	Neg.	Neg	Neg.	
1B	Saline suspension of gut of water strider.	Fed	½ c c	June 4	June 7	Neg				Neg. 2/11/25	
2B	Saline suspension of gut of water strider.	Fed	½ c c	June 4	June 7	Neg				Neg 2/11/25	
3B	Saline suspension of gut of water strider.	Intraperitoneal	½ c c	June 4	June 7	Neg.				Neg 2/11/25	
4B	Saline suspension of gut of water strider.	Intraperitoneal	½ c c	June 4	June 7	Neg				Neg. 2/11/25	
Control 5B					June 7	Neg					
Control 6B					June 7	Neg					
Control No. 1				June 3	June 16	Neg	June 18	Neg.	Neg.		
Control No. 2					June 3	Neg					
Guinea pig No. 169	Saline suspension of 10 16-day cultures of H. ctenocephali.	Intraperitoneal	4 5 c c	June 2	June 3	Neg.	June 16	+(slight)	Neg.		

ATTEMPTED EXPERIMENTAL INFECTION OF MICE

In view of the controversy which has occurred during the past ten years in regard to the possibility of experimentally infecting mice or other laboratory animals either with insect herpetomonads or from cultures of *Leishmania*, a total of thirty-one mice have been inoculated with these protozoans.

A number of box-elder bugs (*Leptocoris trivittatus*) were found to be infected with flagellates. The content of their guts was removed with sterile precautions, and the culture media inoculated. Multiplication of the flagellates occurred in all twenty-four tubes inoculated, but disappeared after four days' inoculation. The remainder of the suspensions of flagellates was inoculated intraperitoneally into three white mice. Each mouse received 1 c.c. of the saline suspension. Five mice were inoculated intraperitoneally with a saline suspension of *Herpetomonas ctenocephali* from cultures. The growth from sixteen ten-day cultures was washed off in saline. Each mouse received 0.7 c.c. of this suspension.

Fantham and Porter (1914) found that *Herpetomonas jaculum* from the hemipteran *Nepa cineria* was especially infective for mice. Consequently, a number of water striders (*Hygrotrechus* sp.) were captured and examined for flagellates. All were found infected and the content of their guts was diluted in saline. Four mice were each fed 0.5 c.c. of the suspension of flagellates. The results of these experiments are summarized in table 5. None of the animals became infected and none of those later skin-tested gave any evidence of being sensitized by the inoculation with living cultures. It is doubtful, however, if mice are anaphylactic.

Recently, Jessner and Amster (1925) have found that dogs infected with *Leishmania tropica* gave a marked reaction to a vaccine prepared from cultural forms. No reaction occurred in healthy animals, or those that had recovered from infection. They state, however, that a definite immunity is established by vaccination.

It was hoped that better results would be obtained by inoculating mice with cultures of *Leishmania*. Row (1924) has recently reported the infection of a mouse by three intraperitoneal inoculations of old cultures of *L. tropica*, which contained only *O* forms as follows:

> 8th November, 1922, with surface cultures of 25th Oct., 1922, containing resistant forms.
>
> 12th January, 1923, with surface culture of 2nd November, 1922.
>
> 22nd January, 1923, with surface culture of 17th October, 1922.

TABLE 6

RESULTS OF THE INOCULATION OF MICE WITH LIVING CULTURES OF LEISHMANIA

No. of mice	Kind of mouse	Method	Strain	Age of culture in days	Date	Amount	Date of death or autopsy	Results of smears at autopsy	Results of cultures at autopsy
2	White	Intraperitoneal	L. donovani (Tunis)	7	5/1/24	1 c.c.	Killed 1/29/25	Neg.	Neg.
2	White	Intraperitoneal	L. donovani (Tunis)	30	5/1/24	1 c.c.	Killed 1/29/25	Neg.	Neg.
1	White	Intraperitoneal	L. brasiliensis	7	5/1/24	1 c.c.	Died 2/7/25	Neg.	Neg.
3	White	Intraperitoneal	L. donovani (Peking)	35	6/18/24	1 c.c.	Killed 6/11/25	Neg.	Neg.
1	White	Intraperitoneal	L. infantum	30	12/17/24 1/29/25	1 c.c. .5 c.c.	Killed 6/11/25	Neg.	Neg.
1	White	Intraperitoneal	L. infantum	30	12/17/24 1/29/25	1 c.c. 1 c.c.	Killed 6/11/25	Neg.	Neg.
1	Peromyscus maniculatus sonoriensis	Intraperitoneal	L. infantum	30	12/17/25	1 c.c.	Died 6/28/25	Neg.	Neg.
2	White	Intraperitoneal	L. tropica	30	12/17/24 1/21/25	1 c.c. 1 c.c.	Killed 6/11/25	Neg.	Neg.
2	Peromyscus maniculatus sonoriensis	Intraperitoneal	L. tropica	30	12/17/24 1/21/25	1 c.c. 1 c.c.	Killed 6/11/25	Neg.	Neg.
2	White	Intraperitoneal	L. brasiliensis	30	12/16/24	.5 c.c.	Killed 6/11/25	Neg.	Neg.
2	Peromyscus maniculatus sonoriensis	Intraperitoneal	L. brasiliensis	25	12/16/24	.5 c.c.	Killed 6/11/25	Neg.	Neg.

Thus his third inoculation was made with a ninety-six day old culture. We have found that even the small round forms (pl. 42, figs. 40 and 41) disappear in cultures of fifty days or older. The inoculation of fourteen white mice by us with both old and young cultures gave equally negative results, as shown in table 6.

Young, Smyly and Brown (1924), working on Kala Azar in China, have found a local field mouse, *Cricetulus griseus,* to be susceptible when inoculated intraperitoneally with *Leishmania donovani* from human cases. We have in this locality a closely related form—*Peromyscus.* Five *Peromyscus maniculatus sonoriensis* were therefore inoculated intraperitoneally with cultures of *Leishmania* (see table 6) with negative results. Thus we have found that even recently isolated cultures of *Leishmania donovani* failed to infect either white mice or *Peromyscus.* This must be due either to the loss of virulence of all of the strains of *Leishmania,* even when cultivated for only a short time, or to the fact that the infective stage in their life cycle has not been found.

SUMMARY AND CONCLUSIONS

1. On N.N.N. culture media both *Leishmania* and *Herpetomonas* pass through a similar life cycle, the division stages of both being a small spherical form. *Leishmania* requires from twenty-one to thirty days to complete its cycle, while only eight to ten days is required by *H. ctenocephali.* All the herpetomonads studied have short flagella never more than the length of the body. The flagella of *Leishmania* are from one to six times the length of the body.

2. Cross-agglutination tests between the *Leishmania* and *Herpetomonas ctenocephali* show no biological relationship to exist between these two genera. Five strains of *Leishmania* all show marked group reactions in the presence of antisera of any member of the genus.

3. Rabbits sensitized to any strain of *Leishmania* give positive skin reactions to antigens of all of the group. No intradermal reaction was obtained with antigens of *H. ctenocephali* or *Trypanosoma lewisi.* Guinea pigs may also be sensitized to both *Leishmania* and *Herpetomonas.*

4. Attempts to experimentally infect mice with cultures of *Leishmania* or *H. ctenocephali* or with flagellates from the guts of insects failed.

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EXPLANATION OF PLATES

Plates 40, 41, and 42 are camera lucida drawings of insect herpetomonads and *Leishmania* from smears fixed in hot Schaudinn's fluid and stained in iron haematoxylin. The magnification in all cases is 3190 diameters.

PLATE 40

All figures of *Herpetomonas ctenocephali*.

Figs 1, 2, 3. Rounded forms predominating in a five day culture (X). 6 × 3 to 3 × 3μ.

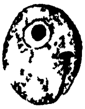
Figs. 4, 5. Large flagellates in twenty-four hour (XI) subcultures from X, 15μ in length.

Figs. 6, 7. Large granular forms in twenty-four hour subculture made from XI after forty-eight hours' incubation at 18° C., 12 to 15μ in length.

Figs. 8, 9. Small slender flagellates and small rounded forms found dividing in a twenty-four hour subculture from XI when seventy-two hours old, 6μ in length.

Fig. 10. Small finely granular flagellates found in small numbers in twenty-four hour subculture from XI when four days old, 11μ in length.

Figs. 11, 12, 13, 14. Rosettes of rounded forms, some dividing, short flagellates also in rosettes and a few solitary, slender, highly vacuolated flagellates. Smears from a twenty-four hour subculture of XI after five days' incubation at 18° C.



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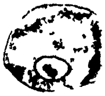

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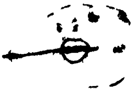

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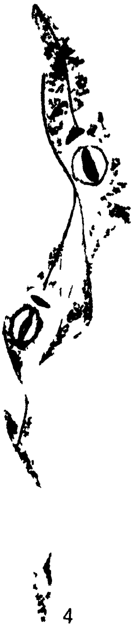

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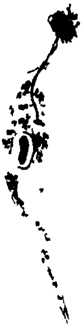

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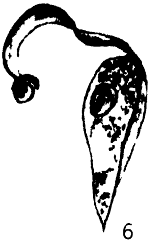

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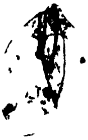

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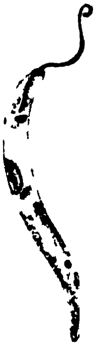

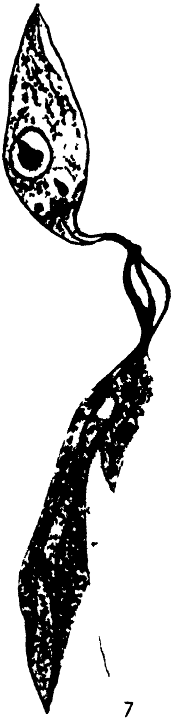

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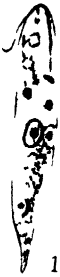

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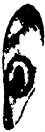

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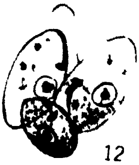

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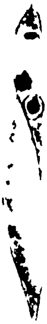

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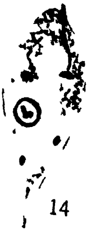

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PLATE 41

All figures of *Herpetomonas ctenocephali* unless otherwise noted.

Figs. 15, 16, 17. Very small, delicate flagellates with a vacuole near the center of the animal body. Occasional small round forms were found dividing. Smear from a twenty-four hour subculture or XI after six days' incubation at 18° C. Flagellates 10μ in length.

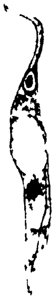
Figs. 18, 19. A few small flagellates and an occasional large plasmodium like body, 13 × 10μ with a single nucleus, blepharoplast and parabasal body very distinct. Smear from a twenty-four hour subculture from XI after seven days' incubation at 18° C.

Figs. 20, 21. Short granular flagellates with heavy nuclei only in smear from twenty-four hour subculture from XI after eight days' incubation at 18° C., 8μ in length.

Figs. 22, 23, 24, 25 Smears from a twenty four hour subculture of XI after nine days' incubation. Many small flagellates 8μ in length and an occasional large solitary flagellate 17μ in length. No round forms found present.

Figs. 26, 27, 28, 29, 30, 31. Stages of flagellates (*Crithidia leptocoridis*) in direct smears from the gut of the box-elder bug, *Leptocoris trivittatus.*

Figs. 32, 33. *Herpetomonas ctenocephali* in direct smear from the gut of the dog flea. These forms were found in rosettes of three or four organisms, 6 × 2.5μ.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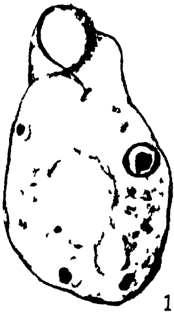
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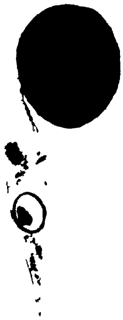
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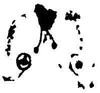
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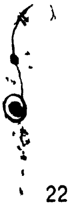
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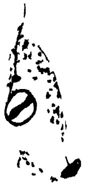
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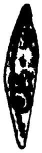
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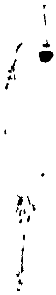
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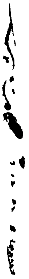
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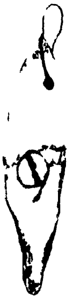
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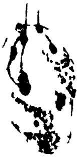
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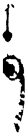
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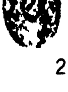
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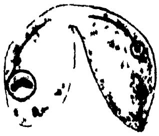
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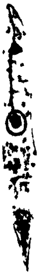
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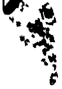
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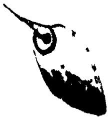
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PLATE 42

All figures of *Leishmania* as noted.

Figs. 34, 35, 36. Stages of flagellates found in a fifteen day old culture of *Leishmania infantum*, 3 to 7μ in length.

Figs. 37. Large flagellate found in thirty day culture of *Leishmania donovani* (Tunis strain), 9μ in length.

Figs. 38, 39. Round, dividing stages found in seven day culture of *L. donovani* (Tunis strain), 5 to 6μ.

Figs. 40, 41. Small round stages, none dividing, in a forty day culture of *L. donovani* (Peking strain), 2.5 × 1.5μ.

Fig. 42. A mass of round forms, several dividing in a fourteen day culture of *L. tropica*, 2 to 6μ in length.

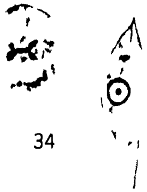
Fig. 43. Type of flagellate found in twenty-three day culture of *L. tropica*, 6μ in length.

Fig. 44. Small round forms only in a forty day culture of *L. tropica.* No dividing forms were found, 3 × 2μ.

Figs. 45, 46. Large flagellates occurring in rosettes in a seven day culture of *L. brasiliensis*, 5 to 10μ in length.

Figs. 47, 48, 49. A round dividing form and slender flagellates in a thirty day culture of *L. brasiliensis*, 4 to 5μ.

Fig. 50. A dividing form in a thirty-five day culture of *L. brasiliensis*, 7 × 4μ.



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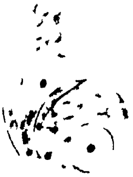

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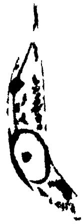

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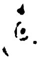

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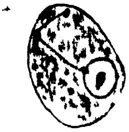

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PLATE 43

Intradermal reactions on guinea pigs sensitized to *Leishmania tropica* and *H. ctenocephali*. Figures natural size and color. Drawings by Dr. Olive Swezy.

Fig. 51. Intradermal reactions on guinea pig 167, forty-eight hours after a sensitizing dose of 5 c.c. of *Leishmania tropica.*

a. Extracting fluid control.
b. Extract of *L. tropica.*

Fig. 52. Normal control pig.

a. Extracting fluid control.
b. Extract of *L. tropica.* No necrosis is present.

Fig. 53. Guinea pig 169, sensitized to *Herpetomonas ctenocephali.*

a. Extracting fluid control.
b. Slight reaction to *H. ctenocephali* antigen.

a *b* 51

a *b* 52

53

a *b*

NOTES ON TERMITES FROM ARIZONA WITH DESCRIPTIONS OF TWO NEW SPECIES

BY

THOMAS E. SNYDER

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NOTES ON TERMITES FROM ARIZONA WITH DESCRIPTIONS OF TWO NEW SPECIES

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THOMAS E. SNYDER

Arizona, from arid desert-like plains to snowcapped mountains, offers a wide range of habitats for termites. In consequence there is a termite or "white ant" fauna of great variety and uncommon interest. Nineteen species and one variety, representing six genera, are to be found in the southern portion of the state; this is nearly one-half the total number of species in the United States, which is 42. Nowhere else in this country are found so many species: In Texas there are 15 species, representing 7 genera; in Florida 12 species, representing 5 genera; and in California 8 species, representing 4 genera. This multiplicity of species is a doubtful distinction, however, since most termites are injurious to the structures and crops of man. Arizona, in time, with a denser population and more intensive cultivation, may find it a serious problem. Professor S. F. Light, upon whose collections this paper is based, found a species of *Reticulitermes*, undetermined in the absence of soldiers, doing extensive damage to the woodwork in the State Capitol of Arizona at Phoenix.

Apparently, some of these termites are rare or of local distribution, and are difficult to discover. The largest species, *Termopsis laticeps* Banks, is present most commonly in the mountains of southern Arizona, and its nearest relatives occur only on the Pacific Coast. In this habitat is also found *Kalotermes occidentis* Walker, the largest and most primitive species of *Kalotermes* known; it ranges south into Lower California. *Leucotermes aureus* Snyder also occurs in these mountains and is the only species of *Leucotermes* found in the United States, its nearest relatives being in the West Indies and in Central America.

One may tramp and collect day after day under a blue, cloudless sky, following up cañons, rimmed in by walls of pink and purple rock, leading from foothills covered with cacti, *Agave*, *Yucca*, mesquite, palo verde, and *Fouquieria* to the higher slopes covered with piñon and Sabino or cedar. Above 7000 feet in the lower stands of yellow pine timber, species of *Reticulitermes* are able to live, but at higher elevations on the mountains in stands of white pine, Douglas fir, and fir and spruce, termites have not been found. Some evening in the last of June, in July, or early August, after you have reached the conclusion that no different termites are to be located, winged adults will be attracted to your camp at the mouth of some cañon, near water, and as they flit about the lights you will probably capture with but little effort, the rarest species of all your arduous collecting.

Fig. 1. Cottonwood root inhabited by both *Kalotermes simplicicornis* Banks and *K. hubbardi* Banks, Copper Butte Cañon, near Ray, Arizona.

Among the foothills and in cañons near water, termites are found in scars on living trees, in dead trees, logs, or stumps (figs. 1 and 2), in the woody stems of cacti (*Opuntia*, fig. 3), and in hardened wounds or woody ribs of the giant cactus (*Cereus*, fig. 4). In the flower stalks and between the rosettes of leaves at the bases of *Agave*, in *Yucca*, and under cow-chips, where there is some moisture, are favorite, although less known, habitats.

Professor S. F. Light, now of the University of California, an authority on Oriental termites, in May, 1925, collected an interesting series of termites in Arizona for the purpose of obtaining living material in which to study the intestinal Protozoa of termites, and these he has kindly permitted me to examine.

In addition to the discovery of two new species and a possible new variety, this collection also extends the known geographical distribution of several species of *Kalotermes* and *Reticulitermes* from southern to central Arizona. *Kalotermes simplicicornis* Banks,

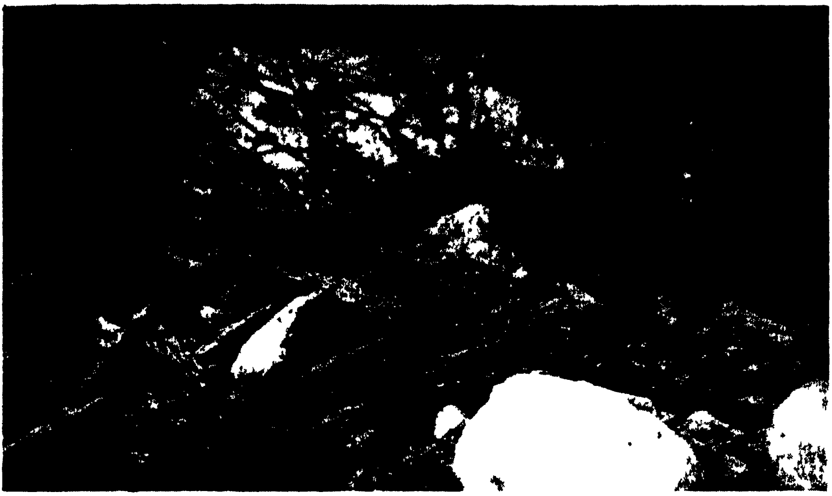

Fig. 2. A dead and partly decayed cottonwood log in which were found both *Kalotermes hubbardi* Banks and *K. simplicicornis* Banks, Copper Butte Cañon, near Ray, Arizona.

Fig. 3. Cactus (*Opuntia fulgida*) that is inhabited by *Reticulitermes hoferi* Banks. Sabino Cañon, Arizona. Photo by George Hofer.

described from Texas but previously found in Sabino Cañon, Santa Catalina Mountains, Arizona, Mr. Light found at Ray, Arizona.

It is possible, when more collecting has been done and winged adults have been correlated with soldiers, that *Kalotermes banksi* Snyder may prove to be the alate form of *K. lighti* sp. nov.; its

Fig. 4. Tree cactus (*Cereus giganteus*) in which are found colonies of *Kalotermes hubbardi* Banks. Sabino Cañon, Arizona. Photo by George Hofer.

size rather indicates this. Likewise, *Leucotermes aureus* Snyder may be the alate form of *Reticulitermes hoferi* Banks; if so, "*Leucotermes*" *hoferi* will suppress *aureus*. While it is difficult to distinguish between soldiers of *Leucotermes* and *Reticulitermes*, the more than usually hairy head and long, straight mandibles of *hoferi* lead one to suspect such a relationship. Possibly a study of the

Protozoa will shed some light on this relationship. *R. hoferi*, described as a variety of *humilis* Banks, is at any rate a valid species, not a variety. More intensive collecting in either Arizona or Texas will undoubtedly uncover new species.

A detailed description of the new species of *Kalotermes*, a list of the termites collected by Professor Light, with the localities where they were collected, and a list of the known species of Arizona termites follows herewith.

Kalotermes arizonensis sp. nov.

Figure 6, 1–3

Soldier.—Head light castaneous-brown, darker anteriorly on slope of epicranial suture, head with mandibles more than twice as long as broad, broader posteriorly than anteriorly, sides concave, with numerous scattered short and fairly long hairs. Eyespot hyaline, small, and subelliptical, close to rim of antennal socket. Postclypeus hyaline and very short. Labrum light castaneous-brown, short, broader than long, tongue-shaped, nearly straight or broadly rounded at apex, where there are long hairs. Gula slender at middle not half as wide as at front.

Mandibles reddish brown at base, elsewhere blackish, broad at base but slender and incurved at apex; left mandible with four small pointed teeth, two on apical and two on basal third; right mandible with two subequal not very large pointed teeth toward the base.

Antennae light yellowish-brown, with ten segments, which become more slender toward apex; third segment dark castaneous-brown, greatly modified, subclavate, elongate, longer than the three following segments together and almost as long as the four following segments; fourth segment shorter but narrower than second; fifth longer than second; last segment elongate, slender and subelliptical.

Pronotum light yellow-brown, darker on margins, not twice as broad as long, anterior margin *not* dentate, deeply and angularly emarginate, sides slope angularly posteriorly, corners not high, posterior margin roundly but shallowly concave, with numerous scattered short and fairly long hairs.

Legs light yellow-brown, short, femora greatly enlarged with long hairs.

Abdomen light yellow-brown, tergites with short hairs and rows of longer hairs near base, cerci fairly elongate, styli present.

Measurements.—

Length	Width
Entire soldier, 8.50 mm.	Head anteriorly, 1.60 mm.
Head with mandibles, 3.95 mm.	Head posteriorly, 1.75 mm.
Head without mandibles, 2.45 mm.	Pronotum, 1.90 mm.
Left mandible, 1.50 mm.	
Hind tibia, 1.20 mm.	
Pronotum, 1.10 mm.	

Type locality.—Copper Butte Cañon, Ray, Arizona.

Described from a single soldier collected at the type locality with an immature soldier of *K. minor* Hagen (?) and nymphs (i.e., young) in gnarled root bases of cottonwood (fig. 5) by S. F. Light in May, 1925.

Holotype, soldier.—Cat. No. 28417, U. S. National Museum.

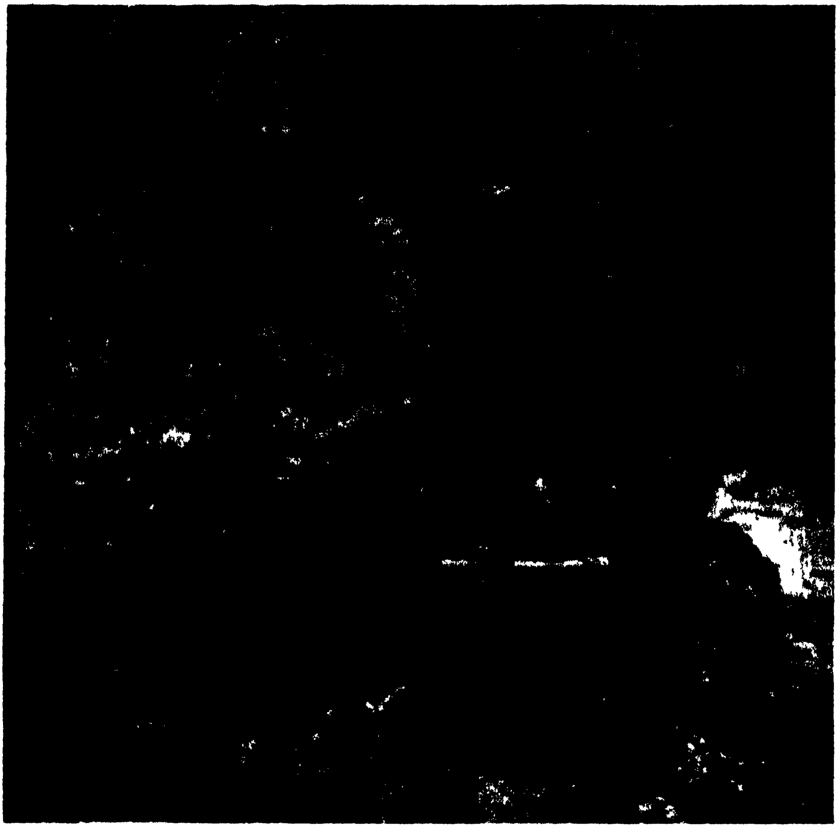

Fig. 5. Gnarled root butts of a cottonwood tree in which were found *Kalotermes arizonensis* Snyder and *K. minor* Hagen, forma *varius* Snyder, Copper Butte Cañon, near Ray, Arizona.

Kalotermes minor forma **varius** f. nov.

The gula of the soldier mentioned above is wider at the center than is normal in *K. minor,* being half as long as at front; antennae with 12 segments, third segment as long as next three together; marginal teeth differ slightly; pronotum not so long in proportion to width; legs with femora greatly swollen. This soldier may represent a new species or variety of *minor;* more material is essential for a complete description.

Kalotermes lighti sp. nov.

Figure 6, 4–6

Soldier.—Head light yellow-brown, darker anteriorly at epicranial suture, where there is a short slope, head with mandibles more than twice as long.as broad, slightly broader posteriorly than anteriorly, lateral margins slightly concave, with few scattered fairly long hairs. Eyespot gray, distinct (suboval), fairly large, on a line with rim of antennal socket. Postclypeus hyaline (white), broader than long. Labrum yellow-brown, tongue-shaped, slightly broader than long, rounded at apex, where there are long hairs. Gula slender at middle, where it is less than half as wide as at anterior margin.

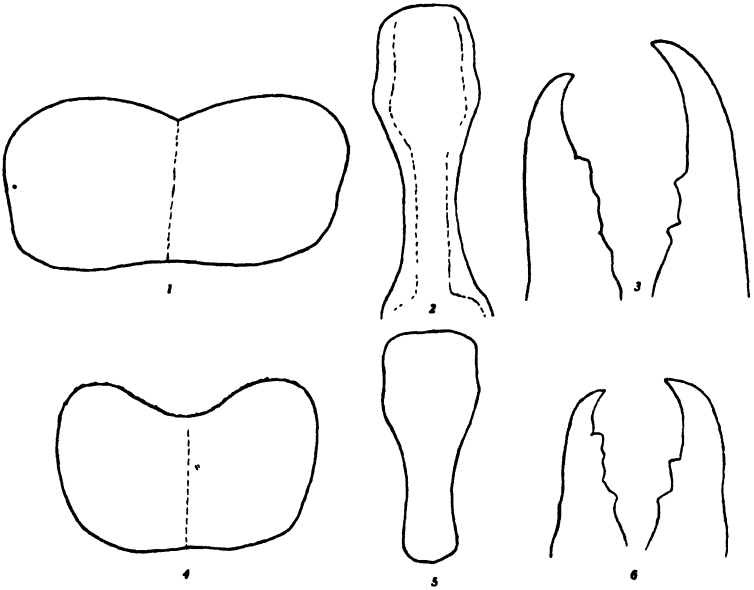

Fig. 6. 1–3, *Kalotermes arizonensis* Snyder, soldier; 1, pronotum, × 22; 2, gula, × 28; 3, mandibles, × 20. 4–6, *Kalotermes lighti* Snyder, soldier; 4, pronotum, × 20; 5, gula, × 20; 6, mandibles, × 32.

Mandibles reddish brown and broad at base, black, slender, pointed and incurved at apex, left mandible with two subequal pointed teeth near apical third and a pointed tooth on basal third of mandible; right mandible with two larger, subequal pointed teeth at basal third of mandible.

Antennae light yellow-brown, with 11+ (? broken) segments, segments become shorter and narrower towards apex, with long hairs; third segment more darkly pigmented, greatly modified, subclavate and longer than fourth and fifth segments together, but not so long as fourth, fifth and sixth together; fourth segment shorter than second or fifth.

Pronotum light yellowish, anterior margin darker and slightly serrate; anterior corners high, rounded and deeply and roundly emarginate anteriorly, sides narrowed posteriorly, posterior margin slightly convex, except medianly where slightly shallowly concave.

Legs white, with tinge of yellow, femora greatly swollen.

Abdomen dirty white with tinge of yellow, tergites with rows of fairly long hairs near base; cerci fairly elongate, styli present.

Measurements.—

Length	Width
Entire soldier, 6.50 mm.	Head anteriorly, 1.30 mm.
Head with mandibles (slightly crossed), 3.00 mm.	Head posteriorly, 1.40 mm.
Head without mandibles, 2.00 mm.	Pronotum, 1.45 mm.
Left mandible, 1.10 mm.	
Pronotum, 0.95–1.00 mm.	
Hind tibia, 1.00 mm.	

Type locality.—Copper Butte Cañon, Ray, Arizona.

Described from two soldiers collected with one nymph of the sexual form at the type locality in May, 1925, by S. F. Light. The colony was in mesquite. This termite is named in honor of Professor Light, who is an authority on Oriental termites. From its small size, it may possibly later prove to be the soldier of *K. banksi* Snyder described in 1920 from the winged forms only, which were collected in Sabino Cañon, Santa Catalina Mountains, Arizona.

Holotype, soldier.—Cat. No. 28418, U. S. National Museum. Professor Light has additional specimens.

LIST OF TERMITES COLLECTED IN ARIZONA BY PROFESSOR S. F. LIGHT

Family KALOTERMITIDAE

Kalotermes arizonensis Snyder.
  Soldier (577 U. S.—Light).
    Ray, Arizona (Copper Butte Cañon), May, 1925 (with one soldier of *Kalotermes minor* Hagen forma *varius* Snyder). In gnarled root bases of cottonwood.

Kalotermes hubbardi Banks.
  Soldiers and nymphs (572 U. S.—Light).
    Phoenix, Arizona, May, 1925. In cottonwood. Numerous colonies.
  Soldiers and nymphs (568 U. S.—Light).
    Sabino Cañon, Santa Catalina Mountains, Arizona, May, 1925. In sycamore and other trees.
  Soldiers and nymphs (573 and 574 U. S.—Light).
    Ray, Arizona (Copper Butte Cañon), May, 1925. In cottonwood.

Kalotermes lighti Snyder.
  Soldiers (576 U. S.—Light).
    Ray, Arizona (Copper Butte Cañon), May, 1925. In mesquite.

Kalotermes minor Hagen.
  Soldiers (571 U. S.—Light).
    Ray, Arizona (Copper Butte Cañon), May, 1925. With *K. simplicicornis* Banks. In cottonwood.

- Deälated adult (573 U. S.—Light).
  - Ray Arizona (Copper Butte Cañon), May, 1925.
- Soldiers and nymphs (569 U. S.—Light).
  - Prescott, Arizona, May, 1925.
- Winged nymphs and soldiers (575 U.S.—Light).
  - Sabino Cañon, Santa Catalina Mountains, Arizona, May, 1925.

Kalotermes minor Hagen forma *varius* Snyder.

- Soldier (577 U. S.—Light).
  - Ray, Arizona (Copper Butte Cañon), May, 1925. In gnarled root bases of cottonwood. (With one soldier of *Kalotermes arizonensis* Snyder).

Kalotermes simplicicornis Banks.

- Soldiers and nymphs (570 U. S.—Light).
  - Ray, Arizona (Copper Butte Cañon), May, 1925. Three colonies; in mesquite butt, cottonwood root, and decaying cottonwood log.

Family RHINOTERMITIDAE.

Reticulitermes hoferi Banks.

- Soldier (565 and 566 U. S.—Light).
  - Ray, Arizona (Copper Butte Cañon), May, 1925.

Reticulitermes humilis Banks.

- Soldiers (567 U. S.—Light).
  - Prescott, Arizona, May 27, 1925. In ash and pine stumps.

LIST OF KNOWN SPECIES OF TERMITES OF ARIZONA

Family KALOTERMITIDAE (9).

- *Termopsis laticeps* Banks.
- *Kalotermes arizonensis* Snyder.
- *Kalotermes banksi* Snyder.
- *Kalotermes hubbardi* Banks.
- *Kalotermes lighti* Snyder.
- *Kalotermes minor* Hagen.
- *Kalotermes minor* Hagen forma *varius* Snyder.
- *Kalotermes occidentis* Walker.
- *Kalotermes simplicicornis* Banks.

Family RHINOTERMITIDAE (6).

- *Leucotermes aureus* Snyder.
- *Reticulitermes claripennis* Banks.
- *Reticulitermes hoferi* Banks.
- *Reticulitermes humilis* Banks.
- *Reticulitermes tibialis* Banks.
- *Reticulitermes tumiceps* Banks.

Family TERMITIDAE (6).

- *Amitermes arizonensis* Banks.
- *Amitermes californicus* Banks.
- *Amitermes confusus* Banks.
- *Amitermes tubiformans* Buckley.
- *Anoplotermes fumosus* Hagen (New record. Collected near Jerome, by Mr. W. W. Jones).
- *Tenuirostritermes tenuirostris* Desneux.

Transmitted January 28, 1926.

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COPELATA FROM THE SAN DIEGO REGION

BY

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BY

CHRISTINE E. ESSENBERG

(Contribution from the Scripps Institution of Oceanography, of the University of California)

CONTENTS

	PAGE
Introduction	399
Material	400
Methods	400
Classification, general morphology, and distribution of Copelata	401
Kowalevskidae	411
1. Kowalevskia	411
Appendicularidae	415
a. Fritillarinae	415
1. Appendicularia	415
2 Fritillaria	418
Description of species	418
b. Oikopleurinae	483
1. Oikopleura	483
Description of species	483
2. Stegosoma	504
3. Megalocercus	507
4 Althoffia	510
Discussion	512
List of species	517
Summary	518
Literature cited	519

INTRODUCTION

When studying, several years ago, the daily plankton collections taken at the Scripps Institution, the author's attention was attracted to the pelagic tunicates, the Copelata, more generally known as the Appendicularia. The results of investigations begun at that time are here presented.

The main object, at first, was to study the behavior of Copelata, especially their reaction to physical and chemical stimuli; identification was considered of secondary importance. But, on account of the delicacy of the animals and their inability to live in an artificial environment for a sufficient length of time, the experimental work was not successful.

The list of recognized species was meanwhile steadily increasing, and this fact led to the present systematic treatment.

Forty-eight species are here recorded from this region, distributed in various genera as follows: *Oikopleura* 9, *Fritillaria* 34, *Stegosoma* 1, *Appendicularia* 1, *Kowalevskia* 1, *Althoffia* 1, *Megalocercus* 1. Thirty of the species are new to science and to these I add eighteen species previously described from other parts of the world.

The author desires to express her appreciation to Dr. W. E. Ritter for his valuable criticisms and for his supervision of the work.

MATERIAL

The material for these studies was obtained from daily collections of plankton taken from a very limited area of water at the end of Scripps Institution pier at La Jolla, near San Diego, California. Additional material was occasionally received from more distant areas and from deeper waters off-shore, but it contributed no additional species. All the species here described have been obtained during a period of several years from the one small area defined above.

These observations thus concentrated have thrown new light on the distribution of this group of organisms. Species recorded from tropical regions, as well as others from higher latitudes and colder waters, appear suddenly and unexpectedly, only to disappear sometimes not to be seen again for several years. The new species here described have, in many instances, been equally sporadic. The observer therefore becomes very skeptical as to some of the statements that have been made concerning the occurrence of certain pelagic marine species in a given locality.

METHODS

On account of the delicacy and complexity of the animals, it is important to be familiar with the technique of handling them. Because of their lack of this knowledge some earlier workers in the field, after spending a good deal of time upon the task, grew discouraged and gave it up as hopeless.

Appendicularians are transparent or semi-transparent when in normal living condition, therefore the organs can be studied as they function in a living animal—a fortunate circumstance, since the identification of species is based largely on characteristics of the internal organs. The spatial relations and outlines of these organs are also easily seen in such specimens. Unfortunately, when dying or dead the organisms become opaque. Hence the animals must be killed quickly and while in active condition. Ten per cent formalin in sea water proved to be one of the most satisfactory killing agents. (The least addition of fresh water tends to make the animals opaque.) For this study a number of hollow glass slides of different sizes have been used. An animal is placed on a slide, and, if it seems necessary, a small drop of iodine is added to the formalin, then a small drop of glycerin. By this process the appendicularian is made most transparent and also slightly stained with the iodine, so that the outline of the various organs can be traced.

By moving the cover glass gently over the hollow in the slide the specimen is rolled over and over, and, by watching, it can be stopped when a desired view is obtained. Sometimes alcohol with turpentine instead of glycerin can be used to advantage as a clearing agent. Attached débris can be removed from the animals by means of a fine camel's hair brush and a fine needle. If the needle is not very smooth, a stiff bristle is preferable.

Classification, General Morphology, and Distribution of Copelata

The historical, evolutionary, and other general matters pertaining to appendicularians have been discussed in detail by Lohmann (1896) and other authors. Observations on seasonal distribution of appendicularians of the San Diego region have been recorded in a previous paper (Essenberg, 1922).

Lohmann's systematic work on Copelata is considered the most complete and up to date, therefore his scheme of classification has been closely followed in the present discussion. Lohmann classified the pelagic tunicates as follows:

Class: *Copelata* Gegenbaur
  Order: *Archipneusta* Lahille
    1. Family: *Kowalevskidae* Lahille
      1. Genus: *Kowalevskia* Fol
    2. Family: *Appendicularidae* Lahille
      1. Subfamily: *Fritillarinae* Seeliger
        2. Genus: *Appendicularia* Fol
        3. Genus: *Fritillaria* Quoy et Gaimard
      2. Subfamily: *Oikopleurinae* Lohmann
        4. Genus: *Oikopleura* Mertens
        5. Genus: *Stegosoma* Chun
        6. Genus: *Megalocercus* Chun
        7. Genus: *Folia* Lohmann
        8. Genus: *Althoffia* Lohmann

All these genera except one (*Folia*) are represented in this report.

CLASS: COPELATA Gegenbaur (1855)

The Copelata are free-swimming, solitary, small-sized tunicates. They differ from the Acopa in the following characteristics, which have been used as criteria of classification: presence of chord; absence of branchial cavity; ventral position of branchial opening of anus; absence of cloaca; formation of cuticle; impossibility of non-sexual reproduction. The Copelata permanently retain the tail with the chord, while the Acopa lose this caudal appendage in their adult stage. On account of their peculiar external appearance, the Copelata were considered for a long time to be the actual larvae of the Ascidiaceae and Thalaceae. They were named by some authors Larvaceae (Herdmann, 1882) and are still to be found in various textbooks under the names of Larvaceae or Appendicularia.

GENERAL MORPHOLOGY

Most Copelata are microscopic in size. They are composed of two main divisions, the trunk and the tail. The length of the trunk varies from 0.4 to 8 mm. The length of the tail is 1.5 to 7 times the length of the trunk. A house or tunica surrounds the animal more or less completely. An epithelium layer forms the body walls. A short endostyle is present in all Copelata except Kowalevskidae. Two branchial openings on the ventral side lead from the floor of the pharynx to the exterior. There is no peribranchial cavity. The digestive system consists of a mouth, pharynx, oesophagus, a one- or two-lobed stomach, an intestine, and a rectum opening on the ventral side. There is no cloaca. A simple heart is present in all Copelata except *Kowalevskia*. All Copelata, except one species, are hermaphroditic.

The tail is broad and long and is attached to the ventral side of the trunk, its width being at right angles to the plane of symmetry of the trunk. The chord occupies the axis of the tail. It is homologous to the spinal chord of vertebrates in embryonic state and consists of a thin, membranous tube closed at both ends and filled with a mass of cartilaginous substance. Inside the membrane are rounded or flattened refractile cells whose function is, supposedly, to secrete the cartilaginous substance which gives the tail elasticity and firmness, so that, after muscular contraction, it springs back to its natural position. These secreting cells show more conspicuously in some species than in others. The caudal nerve extends from the posterior nerve ganglion to the distal end of the tail. It has a great number of ganglia, which give off nerve branches. In some species, the number of nerve cells is constant.

The neural tube is enveloped on both sides by a wide muscular band, which is widest near the center of the tail. These bands are composed of a single layer of striated muscle fibers identical with the muscle fibers of the Vertebrata. They are not in bundles, however, but lie side by side in a single layer and are apparently devoid of a sarcolema. These are the only muscles in the body of Copelata, except the few muscular fibers of the heart.

On the right side of the chord, near the ventral surface of the musculature, are often found the so-called *subchordal* cells, supposedly glandular in nature. They differ in number, shape, and arrangement in different species.

On both sides of the muscular layer of the tail, extend broad, transparent bands of ectothelial structure. The muscles of the tail are held in position between the neural tube and the ectothelium layer by adhesion.

The tail is the chief organ of locomotion. By the contraction of the muscles and with the aid of the elastic chord, the tail is started in an undulating motion and the body thus propelled. During the contraction of the muscles the house surrounding the animal expands, and the tail drives the water into the space thus provided. During the period of rest, the elastic house tends to come back to its natural position, the superfluous water is forced out, and through the contraction of the muscles of the tail, coördinated with the expansion and relapse of the house, locomotion is produced.

The body walls, on the whole, are rigid, giving shape to the body. They are composed of two parts, different in structure and function—a smooth, non-secreting epithelial layer covering the posterior portion of the trunk and the tail, and a secretive, oikoplast, epithelial layer covering the anterior portion of the trunk. The cells of the oikoplast epithelium are large, varying in shape and arrangement, each species having a pattern of its own. Some of the cells near the anterior extremity of the body are unusually large and swollen. The chief function of these oikoplast cells is to secrete the 'house.' The epithelial layer or the outside covering joins the endothelial lining at the oral, branchial, and anal orifices.

The body walls do not have a high resistance for they turn opaque and begin decomposing long before any other signs of approaching death are noticed in the animal. The anterior oikoplast epithelium disintegrates very quickly. One may see an animal with the greater part of its anterior body wall gone, and instead of a mouth there may be left only the huge triangular gap of the open pharynx. At the same time the cilia in the oesophagus and stomach may still be beating vigorously and carrying food particles. The epithelial covering contains glandular cells in various parts of the body.

In some species (the northern) and in some localities the house is said to reach an enormous size. In the San Diego region the house is comparatively small. The author has never seen a Copelata from this locality with a house large enough to cover the entire animal including the tail, although frequently animals have been found which have a small house covering the trunk only.

The house consists of a thick, mucilaginous, clear, colorless substance. It maintains its transparency under almost any circumstances. Neither alcohol, acid, nor any other chemical reagents change its transparency, and very few dyes have any effect on it. The house is thick for the greater part and is decorated with various markings characteristic of the different species. On the dorsoanterior surface two openings, covered with a meshwork, lead to the interior by funnel-shaped projections. A third anterior meshwork is in front of the mouth. Through these openings the water rushes into the cavity, while the larger organisms and the débris are kept outside by the meshes or crossing fibers. The water leaves the cavity through a third posterior opening. This water current supplies the body with food and oxygen and also aids locomotion. If disturbed, the animal slips out of its house and in a few hours the oikoplast cells have secreted a new one. It is probable that a Copelata escapes its enemies in this way, leaving behind only its house.

The nervous system of the Copelata consists of two nerve-ganglia, an anterior and a posterior one, a principal nerve and its branches connecting the two ganglia; and a caudal nerve chord with its ganglia and branches. The principal nerve, after leaving the posterior extremity of the anterior ganglion, sends off a pair of nerves, one to each branchial opening, and then continues its course posteriad on the right dorsal side of the median line, over the dorsal wall of the pharynx and the oesophagus, passing, the median line of the stomach and the heart. Turning then obliquely to the left side of the stomach, it ends in the posterior ganglion. In its course between the two main ganglia the nerve has several small ganglia.

The anterior ganglion is located on the dorsal side behind the mouth between the pharynx and the epithelium. It is pyriform and consists of three parts: fore-, mid-, and hind-brain. The posterior end of the hind-brain attenuates gradually and continues its course posteriad as the principal nerve. Masses of fine nerve fibers arising in the fore-brain run through the dorsal portion of the mid- and hind-brain and join the nerve. The fore-brain is distinct, while the mid- and the hind-brain are more or less fused. The fore-brain sends out anteriad a heavy nerve which soon bifurcates. These two branches may form an arch or a ring around the mouth and send out a number of fine nerves, some of which lead to the pharynx and to the endostyle. while others terminate in cilia-bearing cells surrounding the mouth. These stiff cilia may function as organs of touch and also aid in conveying food particles to the mouth. In some genera two smaller nerves arise from the fore-brain and extend anteriad to the dorsal lip, where they.break up into a number of fine branches ending in refractile cells.

On the left side of the mid-brain lies the spherical otocyst, enclosed between the wall of the mid-brain and a membrane extending from the latter which surrounds the otocyst on its left side. Inside the otocyst, enclosed in a thin membrane, is the otolith, a solid round body composed of interchanging darker and lighter layers (Ihle, 1913). Some investigators claim to have discovered in the dorsal wall of the otocyst a rudimentary eye with a lens (Salensky, 1902).

On the right side of the ganglion is a ciliated groove, the ciliated fossa or organ of smell. It is elongated, running down ventrad and toward the right side, stopping at the pharynx. Its walls consist of simple ciliated epithelial cells. The cilia are very strong, beating rhythmically and simultaneously.

The posterior ganglion is situated on the left side of the median ventral line, near the base of the tail. Its posterior end attenuates into the big caudal nerve which runs along the left side of the neural tube almost the entire length of the tail. The caudal nerve has many ganglia (15–40) varying in number in different genera and species. These ganglia send out fine nerves to the various parts of the tail.

Respiration in Copelata probably takes place all over the surface of the body. The pharynx and the branchial openings, however, may be considered to be the chief organs of respiration. The constantly flowing stream of water in the large, triangular pharynx is separated from the blood only by the very thin membranous wall of the pharynx, and the exchange of gases may take place here. Two short tubes lead from the lateral walls of the pharynx to the exterior, where they terminate in circular branchial openings on the ventral surface of the trunk. These openings are provided with a ring of refractile cells which bear long, stiff cilia. The cilia vibrate in one direction producing a rotatory motion from left to right, causing a rapid flow of water. The current of water usually enters the mouth and leaves the pharynx through the branchial openings, which act in unison. The direction of the current, however, may be reversed, apparently at the will of the animal, whenever it is necessary to expel matter from the mouth or prevent it from entering the digestive tract. The author has occasionally watched an *Oikopleura* holding one end of a spiny *Chaetoceras* in its mouth and keeping the rest of it outside by means of the meshes guarding the mouth. In this situation the current is invariably reversed, entering the pharyngeal cavity through the branchial openings and leaving in a jerky stream through the mouth. This procedure is kept up until the undesired object is removed from the mouth. If at such times a little carmine is added to the water, the water can be seen entering the branchial openings and leaving the pharynx through the mouth. This explains partly why only certain kinds of food are found in the alimentary canal.

The circulatory system is very simple. The heart, in all Copelata except Kowalevskidae, consists of a simple sac, located on the ventral side of the body near the root of the tail. and on the right side of the stomach. The dorsal wall of the heart adjacent to the stomach has striated muscle fibers. Its ventral or outside walls are composed of flat epithelial cells. In some *Oikopleura* the heart consists of two parts separated by a thin membrane (Salensky, 1903). The heart beat or muscular contraction is very rapid, about 3–4 times per second or 200–250 times per minute. The heart beat is supposed to be reversed, as in all Tunicata. However, this reversal of the heart beat, which is so easily seen in other Tunicata, is difficult to see in Copelata.

There are no blood vessels, but only blood sinuses or lacunae through which the colorless blood flows, bathing the internal organs and the body walls. The blood has no corpuscles. Although the blood does not flow in enclosed vessels, nevertheless it follows a certain course. The principal blood flow separates at the anterior end of the body, two currents of blood surrounding the pharnyx in an arch, supplying the pharnyx and branchial tubes with blood, and merging then into one stream. The ventral sinus bathes the endostyle then bifurcates between the two anterior arches, the two streams joining near the anterior nerve-ganglion. Continuing posteriad and following the oesophagus dorsally, it bathes the viscera and genital glands, then descends to the tail along the right side of the chord, and ascends on the left side of the chord to the heart. By the contraction of the muscular wall of the heart, the blood is driven between the walls of the stomach and those of the heart (Ihle, 1913). Fol (1872) thinks that the heart is a thin-walled tube, open at both ends, and that the blood is driven through this tubular heart. Various other authors describe the heart as a closed sac (Salensky, 1902). In the reversal of the heart beat, the direction of blood flow is naturally reversed; i.e., the blood flows in the opposite direction.

The food of Copelata consists of microörganisms, such as small chlorophyll-bearing algae, Naviculacea, some Peridinidae, *Coscinodiscus,* Cystoidae, and various spores and other smooth round microorganisms. Larger spiny organisms, such as *Chaetoceras* and *Rhizolenia,* are never found because the meshes and the complicated system of cilia and flagella guarding the mouth prevent them from entering the alimentary canal.

The walls of the alimentary canal consist for the most part of a single layer of epithelial cells, with a few glandular cells in the walls of the stomach.

The *mouth* is terminal, completely devoid of muscles, rigid, immobile, and constantly open. It is provided with rows or groups of cilia-bearing cells. These cilia differ greatly in size and arrangement in the different species. By their constant rhythmical beat they help to keep flowing the water current which carries the food particles into the large mouth.

The mouth opens into the large, triangular pharynx. The walls of the pharynx extend nearly to the walls of the body. They are thin and membranous with a few nucleated cells. These cells differ in grouping and in function. Some of them produce the thin membranous wall, others are glandular in character, forming the endostyle and the branchial glands; still others bear the vibrating cilia which compose the ciliated bands. These consist of strands or rows of one-layered cells bearing stiff, strongly vibrating cilia. They originate either at the anterior or posterior end of the endostyle, rise obliquely along the lateral walls of the pharynx, and join the dorsal, median band. This arched, ciliated band is flat at its anterior end forming a shallow groove which deepens gradually as it approaches the oesophagus into the walls of which it finally merges. An inferior ventral ciliated band extends from the posterior end of the endostyle, from the ciliated groove to the oesophagus.

The endostyle is back of the mouth on the floor of the pharynx. It is composed of a few large glandular cells. Through the center of the endostyle runs, longitudinally, a ciliated groove, which in some species is a closed canal save for one or two openings. The chief function of the endostyle apparently is to secrete a clear, mucous substance, which is discharged into the pharynx from the anterior end of the endostyle, although some of it enters the pharynx from the posterior end along the inferior ciliated band. By the joint action of the ciliated undulating bands, together with the vibration of the cilia of the endostyle and that of the branchial openings, assisted by the strong current of water, the mucus is twisted and turned into the form of a cord which is conveyed by ciliary action to the oesophagus and thence to the stomach. In this twisted mucus cord the food particles are caught and so conducted to the stomach. Without the ciliated bands and the mucus substance food would leave the pharynx through the branchial openings. All Copelata except Kowalevskidae have an endostyle.

The oesophagus is really the continuation of the narrow posterior end of the pharynx. Its walls are composed of ciliated endothelial cells. They are much thicker than those of the pharynx. The short oesophagus leads into the stomach through which the food is carried by ciliary action.

The shape of the *stomach* and the position of its cardiac and pyloric ends differ greatly in different species. These characteristics are considered important in the identification of species. Lohmann (1896) has based his division of the genera of Oikopleurinae largely on the characteristics and position of the alimentary canal.

The walls of the stomach are thick and are composed of large cells. Some of the cells are glandular in their nature, evidently secreting digestive juices. Others bear strong cilia. Since the stomach is completely devoid of muscles, the cilia are the only means of conveying food to the intestine. The cardiac end of the stomach is on the left side, the pyloric end on the right.

From the pyloric end there extends a very elastic intestine, the walls of which are covered with cilia. In some species the intestine has a few prominent gland cells in the shape of blind sacs. The intestine turns to the ventral side where it empties into the rectum. The rectum is ciliated inside and the anus opens on the median, ventral line. There is no cloaca.

All Copelata, except one species (*Oikopleura dioica*), are hermaphroditic. The reproductive organs usually consist of a single or paired ovary and testis, or one of the two organs may be paired, the other one single. They are usually located in the posterior portion of the body, immediately posterior of the viscera and the point of insertion of the tail. There they begin to develop and to that region they are usually restricted. In some species, however, they spread anteriad over both sides of the trunk in sheets or strands, which more or less completely cover the alimentary canal.

During their development, the sperms are arranged in groups, a number of them being enclosed in a thin membrane which is attached to the body wall by a long *stem*. When the sperm is ripe the membrane bursts open and the sperms escape into the body cavity. This they eventually leave by piercing through the body wall. According to Fol (1872) the sperm matures a few days earlier than the ova. The latter spread out in the body cavity, and finally leave it through the rupture of the body walls.

After the discharge of the sexual products the animal gradually degenerates and dies. In fact, degeneration of other organs begins as the development of the sexual products advances and reaches its maturity. This condition is more noticeable in some species than in others.

GENERAL DISTRIBUTION

The Copelata are of ocean-wide distribution, occurring from equator to both poles, and in depth varying from 0–2000 meters or deeper. Lohmann (1896) through his extensive study of the Copelata has come to the conclusion that these tunicates favor the warm waters and are more numerous in the tropical and subtropical than in boreal zones. The number of species as well as the number of individuals decreases toward higher latitudes and cooler areas. Certain species, however, are more or less restricted to their respective areas. Lohmann has divided the species according to their distribution and tendencies to live within certain limits of temperature, into three groups: (1) species inhabiting only the warm waters on both sides of the equator; (2) species inhabiting only the cold waters, extending to both poles; (3) species found in areas where both cold and warm water species occur. If these areas are subject to strong seasonal changes, the warm-water species are prevalent during the warm season, and the cold-water species are more abundant during the cold season (Lohmann, 1896).

There is, however, a fourth group—the cosmopolitan species—which are of the widest known distribution and are less sensitive to changes in temperature. Some of the most eurytherm species are *Oikopleura longicauda,* which can resist a difference in temperature of 15–16° C.; *Oikopleura fusiformis,* a difference of 17–18° C.; and *Oikopleura dioica,* a difference of 26° C. These species are often found during the summer and fall seasons in latitudes as high as 60°, while *Oikopleura parva,* another warm-water species, has been found in latitudes as high as 81°. When warm-water species are found in higher altitudes, they are usually in depths of 200 meters or deeper. This lower layer of the Gulf Stream is warmer and richer in salts than the upper one. The Copelata can resist a greater difference in temperature when the changes are brought about gradually than when they come about suddenly.

The observations on seasonal distribution of Copelata of the San Diego region show that there is an optimum temperature in which these tunicates are most abundant in the surface waters. With an increase or decrease in temperature there is a decrease of Copelata (Essenberg, 1922).

Lohmann states that the two barriers of distribution are temperature and shallow shore line, since all Copelata, except one species, are inhabitants of the high seas. That the temperature plays an important rôle in distribution is undeniable. But as to the effect of the shallow shore waters, it seems that this might also come more or less directly under the influence of temperature. At any rate, the Copelata have been found in great abundance in this region in the surface waters near the shore. At times they have been even more abundant than on the high seas. This was especially noticeable in 1920 (February to April) when surface hauls were taken simultaneously from a boat on the high seas over a considerable area of the Atlantic and Pacific oceans, and from the pier of the Scripps Institution near shore. The Copelata material obtained in the former catches was very scarce, 0–1 per catch, while in the shore hauls it was abundant, varying from 50 to 300 per catch. On the whole, Copelata are abundant in the surface waters near shore in the San Diego region during the winter months, from September or October to April. They are, however, very scarce or completely absent from the surface waters in this locality during the summer months, between April and September, while off shore about 15 miles, in a depth of 100–200 meters, the Copelata have been found in considerable numbers (40–150 per catch). Various observations of this nature tend to prove that a vertical and horizontal migration of the Copelata takes place. The latter is undoubtedly aided by ocean streams and currents. Thus Aida (1907) has observed that the Copelata are exceedingly rare on the Japanese coast during the cold winter months, but are very abundant there during the summer months when the prevailing south winds are blowing and the Black Current is sweeping near the land. Evidently Appendicularians are carried there by that current, and undoubtedly an upward and downward movement of Appendicularians is taking place according to the season, temperature, food conditions, and other possible factors.

Although Copelata have occasionally been found in depths below 2000 meters, yet they are very scarce below 200 meters. The food conditions below that level are presumably unfavorable for their existence.

It is interesting to note that the arctic and antarctic species show more resemblance to each other than to the species of the warm waters between them. Boreal species from both poles have developed similar adaptational characteristics, most notable of which, and common to species of both polar regions, is the enormously large house (Lohmann, 1905).

Family KOWALEVSKIDAE (Lahille 1888)

Body comparatively short, tail long and narrow; mouth simple, round and large; branchial openings large and ellipsoid; two rows of triangular plates in the walls of the pharynx, extending from mouth to stomach; oikoplast epithelium covers dorsal side of branchial region; hood large; endostyle, heart, and oesophagus wanting. Only one genus with one species known.

Genus **Kowalevskia** (Fol 1872)

The trunk is comparatively stout. Its anterior region is dorsoventrally compressed. The walls of the body are very thin and transparent, resembling more the ectothelium layer of *Fritillaria* than that of *Oikopleura.*

On the dorsal median line of the trunk the cells are very thick. They are arranged in circles forming the conspicuous oikoplast zone, whose function it is to secrete the house. According to Fol (1872) the house of *Kowalevskia* is larger and more delicate than that of any other genus of Copelata. It is about 35 times the size of the animal which secretes it; is ellipsoid in shape, with a smooth outer surface and a single large oval opening leading to the spacious interior.

The mouth is large and round with a few cilia-bearing cells scattered in its outer rim. The large pharynx has two pairs of rows of triangular ciliated plates extending from the mouth to the stomach on both dorsolateral and ventral sides. These plates are longer toward the center opposite the large, ellipsoid branchial openings, decreasing in length toward both ends. Near the stomach they appear as mere rudimentary protuberances. In young animals these platelets are very short and are found only as rudimentary buds in the branchial region. The floor of the pharynx is provided with strong cilia, but there are no ciliated bands on its dorsal and lateral surfaces such as are found in other genera of Copelata.

The platelets with their strongly vibrating cilia seem to be the agents by which the food particles are conveyed in the moving water current to the stomach. These particles are sometimes piled up at the entrance of the stomach, where they accumulate faster than they can be taken care of, while at the same time the water is pure which passes through the branchial openings. It is only when the water is so superabundant that the cilia are unable to hold it all that some of the granules escape through the branchial openings. Near the cardiac end of the stomach, where the ciliated platelets terminate, the pharynx grows narrow, forming a very short, ciliated tube which leads to the stomach. From the walls of this tube long tufts of cilia extend far into the stomach.

The stomach is ovoid in shape. There is no intestine, and the round, pyloric end on the right side opens into the rectum. The walls of the rectum are covered with strongly vibrating cilia. The anus is inconspicuous, and cannot be seen in preserved specimens.

There is apparently no heart in *Kowalevskia*. Since the blood is colorless and devoid of corpuscles, it is impossible to trace its circulation except in an individual having blood parasites. Various authors suggest that the contraction of the tail stimulates the blood circulation. This does not seem to be improbable. The tail is contracted at regular intervals (once in 4 seconds) even though the body remains almost stationary. When held under a cover glass where the animal has the least possible freedom, the undulation of the tail continues. This seems to indicate that the contraction of the tail is not for locomotion alone. It is less frequent, however, than the heart beat in other Copelata (once in 3 seconds). The *tail* is proportionately very long and narrow. Its length is about 7 times the length of the trunk, and the width of the musculature is about double the width of the chord.

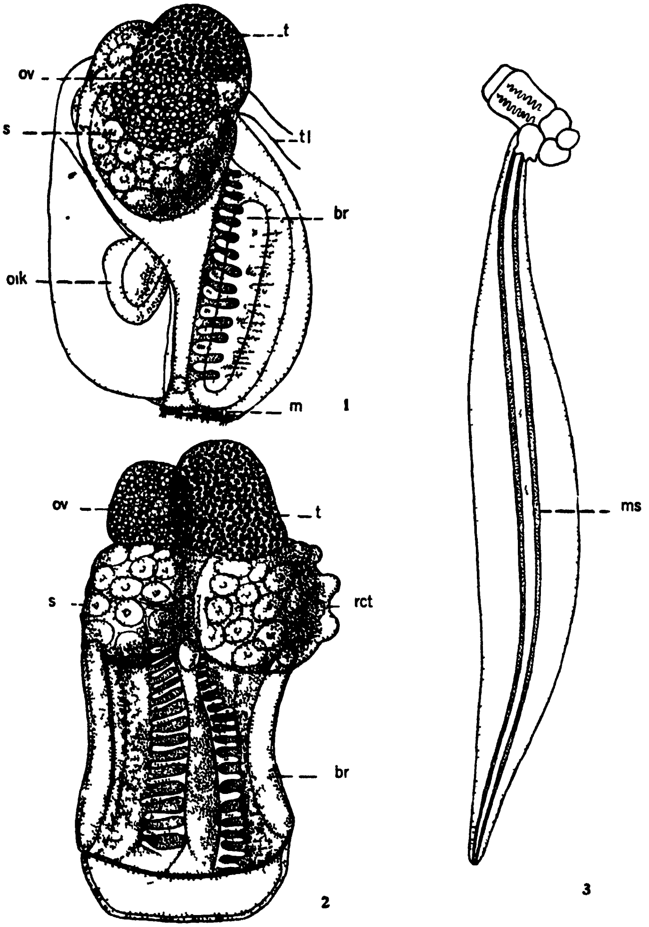
The nervous system in *Kowalevskia* in general is the same as in all other Copelata except that the otocyst, or auditory vesicle, is situated anteriorly and ventrad of the anterior ganglion instead of being on the dorsal left side.

The reproductive system consists of a single round ovary and a round or oblong testis situated in the posterior portion of the trunk.

Kowalevsika tenuis Fol (1872)

Diagnostic characters.—Trunk stout, with its anterior half compressed dorsoventrally; large hood present; mouth simple, large; endostyle, oesophagus, and heart wanting; dorsal and ventral walls of pharynx with two rows of triangular, ciliated plates; stomach round, with its pyloric end leading directly into the rectum; tail long and pointed with narrow musculature.

Kowalevskia tenuis is a medium-sized species. The anterior portion of the trunk is dorsoventrally compressed. A large hood arises from the dorsal side of the gastro-genital region and extends anteriad covering the dorsal surface of the branchial region (fig. 1). The oikoplast epithelium covers the median dorsal side of the anterior portion of the trunk, forming a large ventral process. This process constitutes the oikoplast zone (fig. 1, *oik.*) composed of large secreting cells. The peculiar shape of the oikoplast zone is characteristic of this species only.



Figs. 1–3 *Kowalevskia tenuis* Fol.

Fig. 1. View of left side of trunk. × 160. Fig. 2. Ventral view of trunk. × 160. Fig. 3. The whole animal. × 20. Abbreviations: *br.*, branchial openings; *m.*, mouth; *m.s.*, musculature of tail; *ov.*, ovary; *oik.*, oikoplast zone; *rct.*, rectum; *s.*, stomach; *t.*, testis; *tl.*, tail.

The mouth is large with a ring-shaped unbroken lip. The rim of the lip is provided with fine cilia (fig. 1, *m.*). In addition to these there are a few tufts with long cilia originating from large refractile cells.

The endostyle and the ciliated bands, characteristic in all other species of Copelata, are wanting in *Kowalevskia tenuis.* The floor of the large pharynx is covered with cilia. From its dorsal and lateroventral walls arise two rows of somewhat triangular, ciliated plates, extending from mouth to stomach. They undoubtedly take the place of the ciliated bands of other species in conveying the food particles to the stomach. The branchial openings are ellipsoid and unusually large (figs. 1, 2, *br.*). The branchial openings elongate and become proportionately larger as the animals grow older.

There is no real oesophagus but the large pharynx becomes narrower near the stomach and is covered on the inside with long cilia which extend far into the stomach. The stomach is almost round, situated on the left side. Adjacent to it on the right side is the large rectum. The walls of the stomach are composed of large cells (figs. 1, 2, *s.*). There is no intestine, but the pyloric end on the right side of the stomach empties directly into the rectum (fig. 2, *rct.*). The very inconspicuous anus is situated on the right ventral side of the rectum.

Apparently no heart is present in *Kowalevskia tenuis.* The otocyst is located on the anterioventral side of the anterior ganglion and shows more conspicuously in some individuals than in others.

The reproductive organs consist of a single spherical ovary and an elongated testis. They are located posteriad of the digestive organs (figs. 1, 2, *ov.*, *t.*).

The tail is long and narrow, with a narrow musculature about 1.5 the width of the chord (fig. 3, *ms.*). The peculiar undulating motion of the tail is so different from that of any other Copelata that it may be considered as a distinguishing characteristic of the genus *Kowalevskia.*

The length of the trunk varies in different individuals from 0.5 to 1 mm. The width of the trunk is about 0.5 of its length. The length of the tail is about 7 times the length of the trunk.

The species is very rare in the San Diego region. Up to the present time only seven specimens have been found, all in the surface catches at the pier. They were found on the following dates:

KOWALEVISKIA TENUIS

Date	Temperature*	Number
December 14, 1920	13°94	1
December 16, 1920	13°95	2
December 17, 1920	13°80	1
December 18, 1920	14°18	1
February 11, 1922	13°70	2

* All temperatures throughout the paper are given in degrees Centigrade.

The average temperature at which *Kowalevskia tenuis* has occurred here is about 14° C. Since the species has appeared only sporadically, however, too much significance should not be attached to the data pertaining to temperature. In addition to the daily collections from the pier, numerous surface catches have been examined from other parts of the ocean, as well as catches from depths of 150 meters, taken 10–15 miles off shore near the Scripps Institution during the months of June and July, 1921, but no *Kowalevskia tenuis* have been found. Aida

(1907) found it a frequent visitor of the Japanese coast waters, where it usually occurred simultaneously with *Noctiluca.* Fol (1872) found it in abundance in the Mediterranean Sea near Messina, where it occurred daily from April until June and then disappeared completely. Lohmann (1896) reports its distribution in the warm surface waters of the Atlantic Ocean from near the equator to the coast of Portugal; between Bermuda Islands and Cape Verde; and near the shore of Guinea. The temperature is not mentioned by any of the observers.

Family APPENDICULARIDAE

Subfamily FRITILLARINAE Seeliger (1895)

Body slender, with few exceptions. Tail proportionately short and wide. Mouth simple, round, without a curved underlip. In many cases it has long projecting lobes bearing tactile cells and cilia or flagella. Oesophagus runs horizontally, opening anteriorly in the simple round stomach, whose walls are formed of large glandular cells. The intestine often has round accessory bodies and leads into a more or less prominent rectum. A more or less conspicuous heart is always present. Two branchial openings on the ventral surface are immediately posteriad to the endostyle. Endostyle made up of few large cells, in many species both its ends being turned dorsally, otherwise the anterior end is very broad with one known exception where the reverse is true.

The subfamily Fritillarinae has two genera: *Appendicularia* and *Fritillaria* distinguished as follows: *Appendicularia:* without hood; house large, may cover the whole animal; body short; mouth simple, round, without projecting lobes; rectum enormously large, pear-shaped; caudal fins of medium width.

In genus *Fritillaria* a hood is always present; house small, body long; mouth in most species with projecting lobes bearing tactile cells and flagella; rectum small; tail short and wide. The very wide caudal fins at the proximal end are suddenly reduced to a very narrow rim.

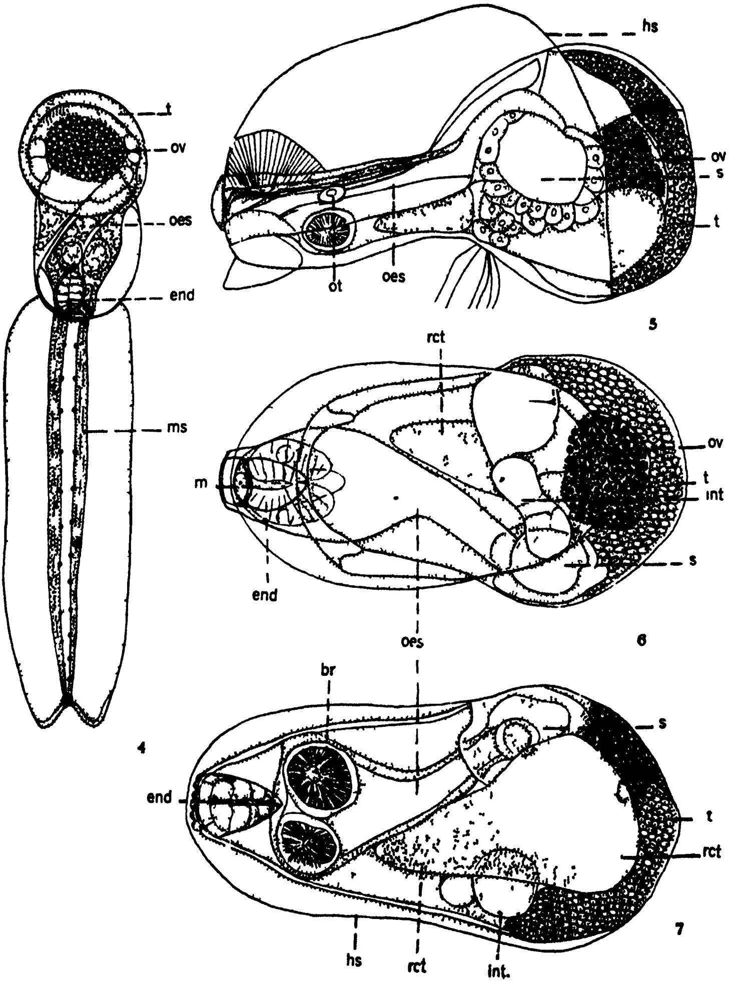
Genus **Appendicularia** Fol (1874)

Appendicularia sicula Fol

Diagnostic characters.—Body short, with its anterior half greatly depressed and its posterior portion bulging; no hood; mouth simple, round; endostyle short, slightly curved; oesophagus opens into the left side of the stomach; stomach round; intestine elongated; rectum enormously large; tail of medium width, more than twice the length of the body.

Appendicularia sicula is a small species. The trunk is short with its anterior portion very narrow, dorsoventrally compressed, while its posterior half is greatly expanded, almost ball-shaped (fig. 5). The oikoplast layer extends far over the posterior region of the trunk, covering most of the alimentary canal and part of the reproductive glands.

The mouth is simple, round (fig. 6, *m*), with its exterior ridge covered with a row of cilia-bearing cells. These cilia are so fine that they can be discerned only under high magnification, in living animals, and while in action. They probably are concerned with the sense of touch and also aid in driving food particles into the mouth. The endostyle is short, consisting of a few large cells (figs. 6, 7, *end.*). The otocyst is small (fig. 5, *ot.*). The large circular branchial openings are located immediately posteriad to the endostyle, without touching the latter (fig. 7, *br.*).



Figs. 4–7. *Appendicularia sicula* Fol.

Fig. 4. Dorsal view of whole animal. × 80. Fig. 5. View of left side of trunk. × 160. Fig. 6. Dorsal view of trunk. × 160. Fig. 7. Ventral view of trunk. × 160. Abbreviations: *br.*, branchial openings; *end.*, endostyle; *int.*, intestine; *hs.*, house; *ms.*, musculature of tail; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis.

The oesophagus is wide at its anterior end gradually becoming narrower toward its posterior end. It turns, making a horse-shoe-shaped loop around anterodorsal side of the stomach, opening then into the dorsoposterior side of the latter (figs. 5-7, *oes.*). The stomach is round. Its thick walls are composed of large glandular cells (figs. 5, 6, *s.*). The intestine is comparatively long, extending across the dorsal side of the body from left to right, ending in the right side of the rectum (figs. 6, 7, *int.*). The pear-shaped rectum is enormously large, and is always filled to its greatest capacity with dark microörganisms, thus making the study of other organs, in some cases, extremely difficult and to some extent impossible (figs. 6, 7, *rct.*).

The heart is located between the stomach and the large rectum. When a living animal is observed while in a most favorable position, the heart is noticeable by its rhythmical contractions. Any detailed study of this organ has been impossible on account of the large rectum and reproductive organs which cover it, thus obscuring it from view.

The reproductive organs consist of a round ovary located on the dorsal side of the posterior end of the trunk. The ovary is partly surrounded by the long sausage-, or horse-shoe-shaped testis. The latter occupies the extreme posterior end of the trunk, partly surrounding the ovary and the upper end of the rectum, forming a semicircle extending anteriad laterally and dorsally (figs. 4, 6, 7).

The tail is uniform in width, bifurcated, with its distal end covered with cilia. The musculature is about twice the width of the chord. A number of cells are visible in the chord (fig. 4).

Appendicularia sicula is a small species. The length of the trunk is about 0.4 to 0.8 mm. The length of the tail is about 2 to 3 times the length of the trunk, the width about 0.33 of its length.

This species is easily recognized by its enormously large rectum, which is always filled with dark brown microörganisms, giving the animal an appearance of a swift-moving, dark object. It is very active, moving constantly, and vibrating its tail rapidly.

The species is poorly represented in this region, appearing at very irregular intervals and in very limited numbers. It may not be observed for a year or longer and then appear in one or more catches in succession only to soon disappear again. In the period of nearly five years only twenty specimens have been found, on the following dates:

Appendicularia sicula

Date	Temperature	Number
November 2, 1917	16°7	1
November 8, 1917	16°.5	2
December 1, 1917	15°.7	1
December 4, 1917	15°.9	1
December 13, 1917	15°.7	1
December 14, 1917	15°5	2
November 20, 1918	16°.3	1
December 4, 1920	14°.31	3
December 5, 1920	14°.1	1
December 10, 1920	14°.3	2
December 1, 1921	15°.17	1
December 2, 1921	14°.98	1
December 12, 1921	15°.3	2
December 13, 1921	14°.94	1

The above data point to the conclusion that *Appendicularia sicula* occurs occasionally in the surface waters of the San Diego region during the months of November and December. They are probably there during January, also, but, if so, have escaped my attention. Considering the fact that surface hauls are taken from a very small and limited area at the pier, it may be that they are caught in the net only when very abundant. They are not easily discovered when dead or motionless among an abundant number of other organisms. Fol (1872) also noticed that *Appendicularia sicula* sometimes disappeared for a year from the waters of Messina. According to Lohmann (1896), *Appendicularia sicula* has been found in the surface waters of the warm seas, occurring regularly between Cape Verde and the Guinea stream from August to September. Near the shores of Brazil it was found from September to October. In September it was very abundant near the mouth of the Amazon River and near the shores of Sierra Leone in Africa. Fol (1874) found it in the Mediterranean Sea near Messina in May, and Langerhans (1880) reports its occurrence near Madeira. Lohmann (1896) has also reported it from the Indian Ocean and the Canal of Zanzibar. In the latter locality it was found from the last part of November to the beginning of March. The temperature of the surface waters in which this appendicularian was found varies from 11°0 to 29°5 C. Since in many places vertical hauls were taken in an open net at depths from 0 to 20 or 200 meters, the appendicularians might have come entirely from lower, cooler regions far below 29° C. temperature.

Genus **Fritillaria** Fol (1872)

The genus *Fritillaria* is easily distinguished from other genera of Copelata by its slender trunk, which is more or less constricted in the center where the tail is attached. The anterior portion of the trunk contains the mouth, pharynx, and oesophagus; the median portion, the heart and stomach; the posterior portion, the genital organs.

The oikoplast epithelium is confined to the extreme anterior end of the trunk. Posterior to it arises a fold extending anteriad and forming the hood characteristic of the genus. The hood covers the dorsal and lateral sides of the anterior portion of the trunk.

The mouth varies greatly in shape in different species. In most cases it is provided with long projecting lobes varying in number and arrangement, but it may be simple and round. The mouth is always surrounded by large cells, bearing cilia and cirri, sometimes arranged in a very complicated pattern.

The pharynx is wide but somewhat dorsoventrally compressed due to the peculiar oikoplast formation. The endostyle is composed of a few large secreting cells and in most cases both of its ends are turned up or dorsad, but it may be straight. Its function is to secrete the mucus substances in which the food is conveyed to the stomach. Immediately posterior of the endostyle are the branchial openings.

The oesophagus is large and strongly ciliated. The cilia are unusually long near the entrance of the stomach extending far into it and beating simultaneously to produce an undulating motion. The stomach is usually round. Its walls are composed of large nucleated cells and are covered interiorly with fine cilia. A short intestine arises on the right side from the pyloric end of the stomach leading to the rectum, which is situated on the right side of the stomach. The walls of the intestines are thin, ciliated inside, and capable of great expansion. The intestine is often accompanied by two or more large glandular cells situated on its dorsal and ventral sides.

The nervous system, in general, is the same as in all Copelata.

The heart is situated on the ventral side of the oesophagus and on the ventral and left side of the stomach. Its muscular fibers usually extend transversely across the body.

The reproductive organs occupy the posterior end of the trunk. They consist of an ovary and testis, both of which may be single, or either one or both of them may be paired. They vary in size, shape, and relative position, but in most cases the ovary is round, situated immediately posterior to the digestive system, and followed by the posteriad-extending testis. The latter is usually attached to the body walls by a number of fine filaments.

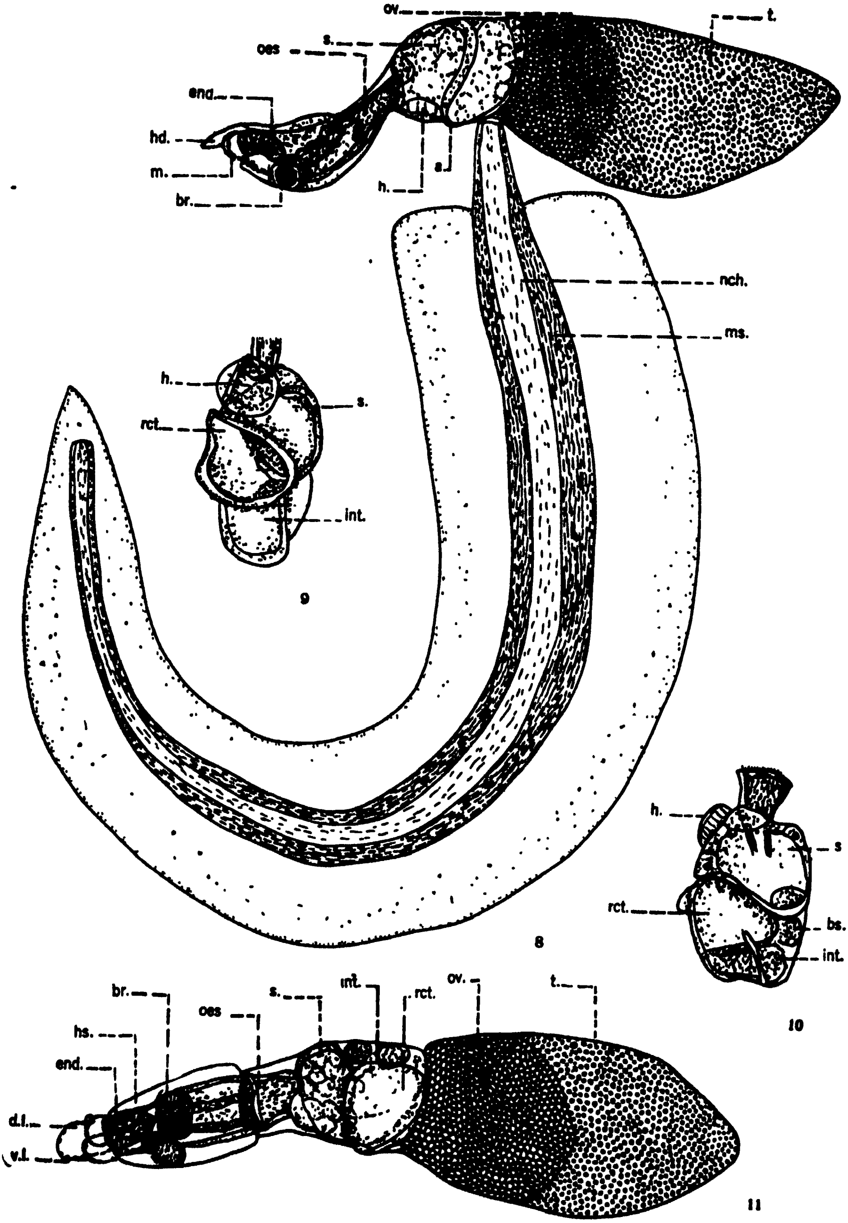
The tail is comparatively short. Its length usually is 1.5 times the length of the trunk. The musculature is narrow, in most cases hardly surpassing the width of the chord. The outer, transparent caudal layers are very wide, spreading out like wings. The distal end of the tail is often bifurcated.

The genus *Fritillaria* is very widely distributed and contains more species than the total number of species of all other genera of Copelata. In the San Diego region, 34 species of *Fritillaria* were found. They are exclusively winter visitors, almost never occurring here in the summer season between April and September.

Fritillaria tenebra, sp. nov.

Diagnostic characters.—Trunk narrow and pointed at both ends. In older, mature animals posterior portion of trunk largely expanded by bulky gonads; endostyle slightly curved; oesophagus short and wide; stomach connected with the pyramidal rectum by the narrow intestine; tail long and pointed (fig. 8).

The mouth is surrounded by one dorsal and two ventral lips, the latter overlapping in the center (fig. 11, *v. l.*). The margins of the lips bear a number of small refractile cilia-bearing cells. The medium-sized endostyle is slightly curved and consists of a few large cells (fig. 11, *end.*). The branchial openings are small and circular (figs. 8, 11). The branchial tubes are short, slightly bent, and the inside openings show faintly at the same time and at the same focus as the outside ventral ones. They are located immediately posteriad to the endostyle. Two rows of large cells extend along the ventral surface of the pharynx from the endostyle to the base of the anterior portion of the body (fig. 11).



Figs. 8–11. *Fritillaria tenebra* sp. nov. × 160.

Fig. 8. View of left side of whole animal. Fig. 9. Ventral view of the alimentary canal. Fig. 10. Dorsal view of the alimentary canal. Fig. 11. Ventral view of the trunk. Abbreviations: *a*, anus; *br.*, branchial openings; *bs.*, blind sac; *d.l.*, dorsal lip; *end.*, endostyle; *h.*, heart; *hd.*, hood; *int.*, intestine; *m.*, mouth; *nch.*, notochord; *oes.*, oesophagus; *ov.*, ovary; *s.*, stomach; *t.*, testis.

The oesophagus is short, wide, and strongly ciliated (fig. 11, *oes.*). The stomach is dorsal in position with large cells composing its walls. These cells are especially conspicuous in the anterior portion of the stomach wall (fig. 10). The stomach is angular with the anterior end narrower and the right corner at the bottom slightly longer. The intestine starts from the right basal end of the stomach, runs dorsally covering a portion of the rectum, then bends, entering the rectum from the posterior side (figs. 9, 10, *int.*). It is armed on both sides by a pair of blind sacs (fig. 10, *bs.*), or glandular cells. The rectum is irregularly pear-shaped, being too wide at the bottom to be exactly pear-shaped. The heart is large, occupying part of the anterior portion of the body and covering part of the oesophagus (figs. 9, 10, *h.*). Almost half of the length of the body is occupied by the large, bulky gonads. The ovary is large, nearly spherical, covering partly the posterior part of the digestive system (figs. 8, 11, *ov.*). The testis follows the ovary posteriad, partly surrounding and overlapping it, and gradually tapering toward the posterior end.

The tail is long and broad, its wings sharply truncate anteriorly, its posterior end long-pointed (fig. 8). Its musculature is narrow toward both ends, greatly increasing in width in the middle region. The notochord is almost uniform in width throughout its entire length.

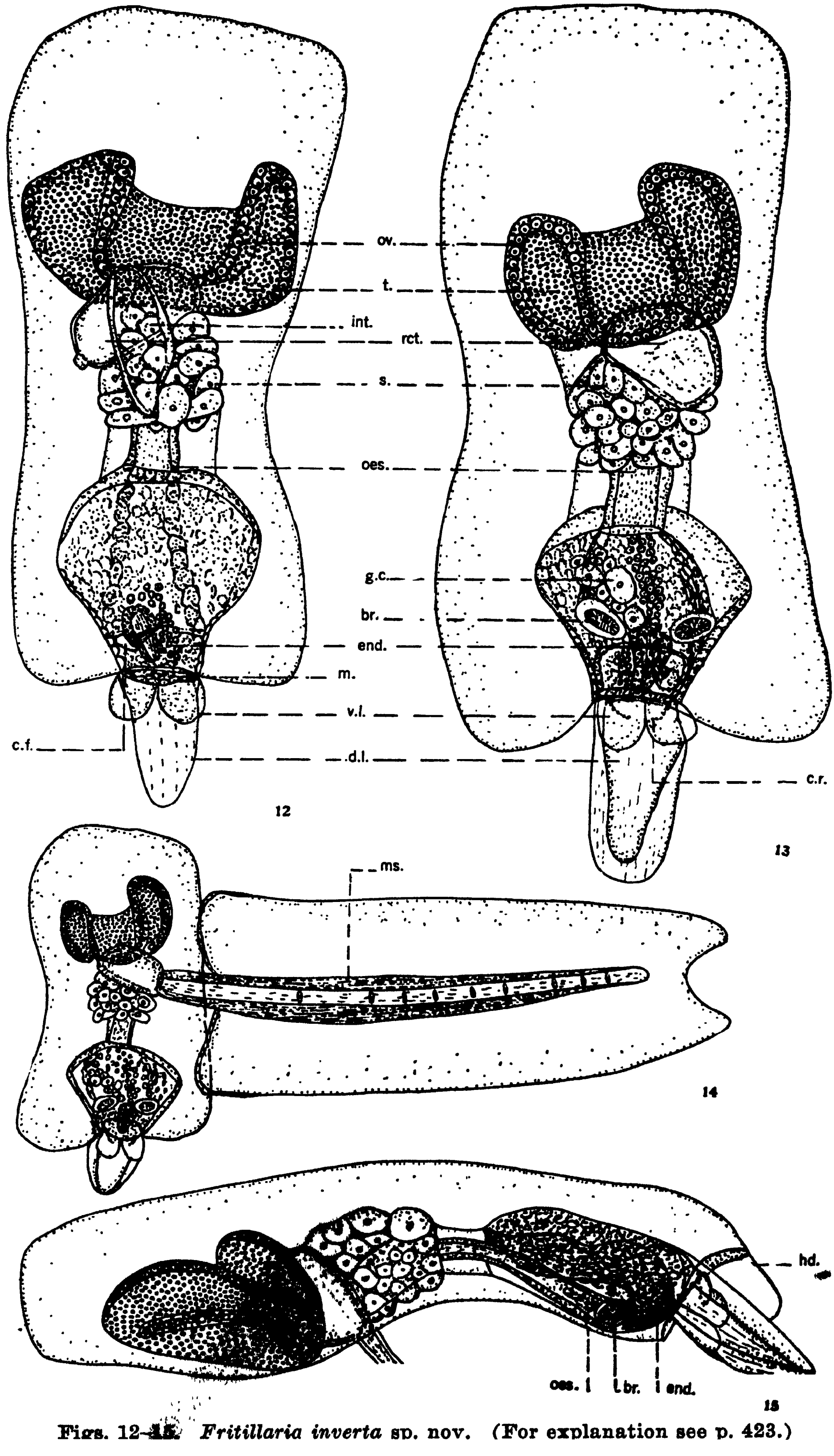
The anterior portion of the body is usually slightly bent ventrad. The length of the body is about 0.54 mm. The width is about 0.16 of its length, measured across the region of the stomach. The length of the tail is about 2.5 times the length of the body, the width about 0.17 of its length. Its musculature in its widest portion is about twice the width of the notochord and about 0.25 of the entire width of the tail.

This species is frequently found here during the winter months between November and March. It was taken on the following dates:

Fritillaria tenebra

Date	Temperature	Number
January 3, 1919	14°5	1
January 7, 1919	14°.5	1
December 3, 1919	15°.06	1
December 15, 1919	14°.24	3
December 16, 1919	14°.11	2
December 22, 1919	14°.3	1
March 15, 1920	13°.4	1
December 3, 1920	15°.12	1
December 5, 1920	15°.7	3
December 11, 1920	14°36	1
February 7, 1921	14°.5	1
February 27, 1921	13°.4	3
December 12, 1921	14°.5	2
March 24, 1922	14°.6	1

As the data indicate, *Fritillaria tenebra* has been found here at an average temperature of 14°.5.



Figs. 12–15. *Fritillaria inverta* sp. nov. (For explanation see p. 423.)

Fritillaria inverta sp. nov.

Diagnostic characters.—A large species. Trunk broad and angular when seen from either dorsal or ventral side; hood prominent; dorsal lip long and wide; two ventral lips medium-sized; ciliated fossa conspicuous; anterior end of endostyle narrow, widening toward posterior end; ovary in strands or coils winding over the large testis.

Fritillaria inverta greatly resembles *F. bicornis* Lohmann. The chief differences lie in the shape of the tail and the endostyle. The latter organ appears in the reverse position in the two species. The endostyle in *F. inverta* is like that in *F. bicornis* changed end for end (figs. 12, 13, 15, *end.*). The tail of *F. inverta* is somewhat more tapering and the terminal notch is rounder and deeper than that of *F. bicornis.*

The mouth is surrounded by a very long dorsal lip and by two medium-sized ventral lips (figs. 12, 13). Above, on each side of the mouth is a semicircle of small cells characteristic of the cilia-bearing cells in other species of *Fritillaria* (fig. 13, *c.r.*). The endostyle is large and unique in shape, with its anterior end narrow, gradually increasing in width toward the posterior end, terminating in a rounded, posterior tip (figs. 12, 13, *end.*). This is the only species of *Fritillaria,* as far as I know, that has an endostyle of this shape. In all other species the anterior end is wider than the posterior one. The endostyle consists of a few large cells of which the two anterior ones are considerably larger than the others. Adjoining the posterior end of the endostyle and extending obliquely dorsad are a group of large cells (fig. 13, *g.c.*), similar to those found in *F. venusta.* A conspicuous ciliated fossa crosses the endostyle obliquely as seen from above, in the dorsal view, its vibrating cilia being borne by a group of cells (fig. 12, *c.f.*).

The branchial openings are comparatively small, oval, situated on a level with the endostyle and immediately posteriad to it (fig. 13, *br.*).

The oesophagus is broad and short. The stomach is cuboid in general shape but is irregular on account of the large protruding cells. The intestine is large and straight, and is situated on the dorsal right side of the stomach and squarely across the rectum, into which it opens. In about the middle of the intestine are a few cells protruding, on both sides, from its walls. The rectum is narrow and long, lying squarely posteriad to the stomach (figs. 12, 13, *int.*, *rct.*).

The gonads in this species are different in shape from those of the majority of other species of *Fritillaria,* but are similar to those of *Fritillaria bicornis.* They are short and wide, with two prominent posterior corners. The ovary consists of narrow strands coiling over the large, curved, sausage-shaped testis (figs. 12, 13, 15, *ov.*, *t.*). The animal is sac-shaped due to the large cuticle that extends far beyond the posterior end of the gonads and gives the animal, as a whole, a bulky angular appearance.

Fig. 12. Dorsal view of trunk. × 80. Fig. 13. Ventral view of trunk. × 80. Fig. 14. Ventral view of entire animal. × 40. Fig. 15. View of right side of trunk. × 80. Abbreviations: *br.*, branchial opening; *end.*, endostyle; *c.f.*, ciliated fossa; *g.c.*, group of cells; *c.r.*, ring of cells; *d.l.*, dorsal lip; *hd.*, hood; *int.*, intestine; *m.*, mouth; *m.s.*, musculature; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

The tail is broad, deeply and roundly bifurcated at the end, and decreasing but little in width distally (fig. 14). The musculature of the tail is narrow (fig. 12, *ms.*). The chord is uniform in width with a few cells scattered in it corresponding to the "*Muskelkerne*" in *F. fraudax* described by Lohmann.

Fritillaria inverta is a large species. The length of the body is 1.6 mm. The width is about 0.33 of its length measuring from side to side. The length of the tail is 1.4 times the length of the body. The width of the tail is about 0.33 of its length. The width of the musculature is about 0.2 of that of the tail.

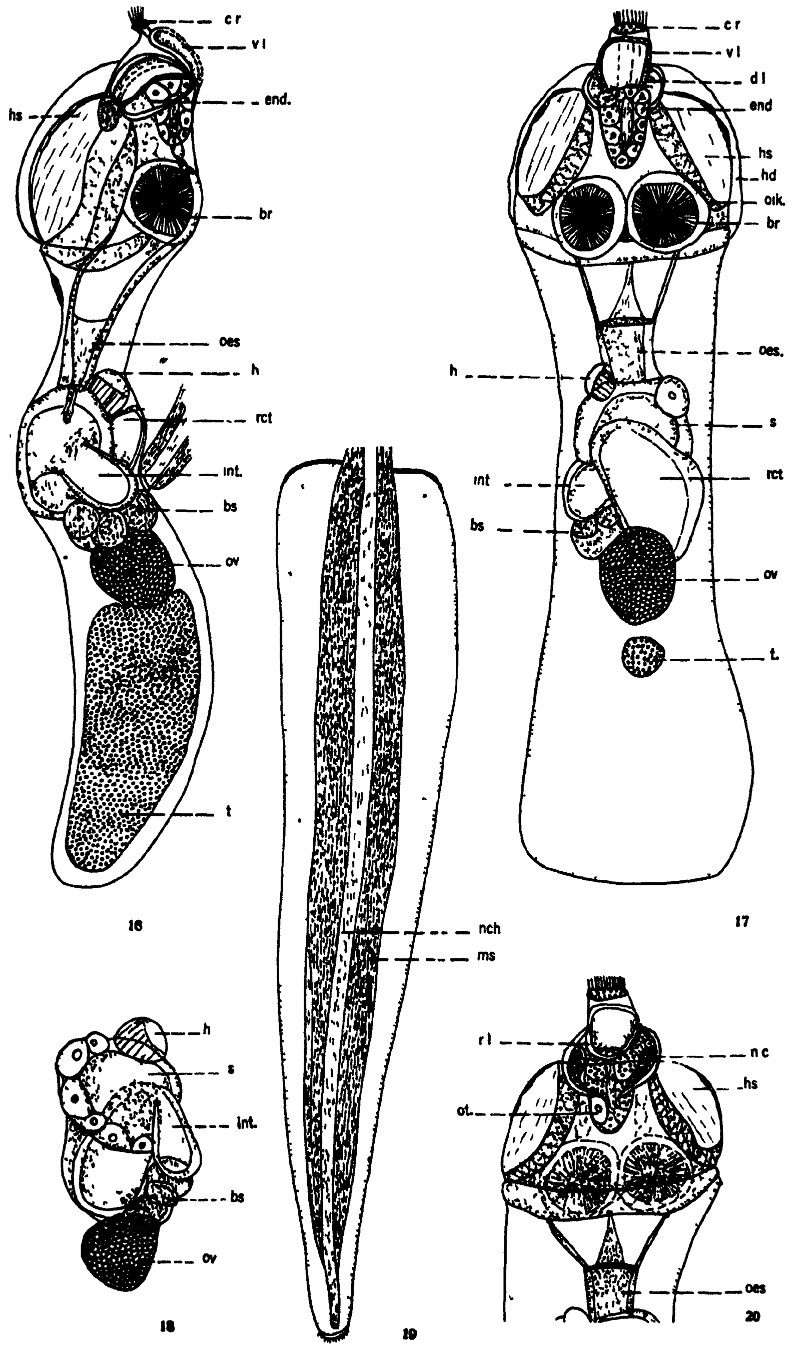
The species is rare in this vicinity. Only two specimens have been found thus far. One of these was taken in July, 1921, in a vertical haul from a depth of 0–200 meters, 10 miles off shore, at a surface temperature of 20°2. The other was found in December, 1921, in the surface waters at the pier of the Scripps Institution when the temperature was 15°6.

Fritillaria lohmanni sp. nov.

Diagnostic characters.—A small species. Trunk sac-shaped, almost uniform in width, but with its posterior end broad and convex; dorsal lip absent; ventral lip single with long cilia; endostyle broad; branchial openings very large; tail broad, tapering very gradually toward the end and terminating in an obtusely rounded tip.

Thick, curved ridges surround the mouth laterally and dorsally (fig. 20, *r.l.*). They are similar to the corresponding structures in *F. gracilis*. These ridges project anteriad, becoming sharp peaks with a refractile body on each (fig. 20). Evidently they are connected with cilia, since they have all the appearance of similar bodies in other species which possess cilia. From these two peaks the ridges run downward in a horse-shoe-shaped curve. The inside curve is surrounded by a larger semicircle making a sharp angle at the two projecting peaks, bulging out laterally and meeting in a semi-circle on the dorsal surface. Inside of the outer ridges, on the dorsal surface, are two oblong refractile structures, one of which meets the otocyst (fig. 20, *n.c.*). The above described arches may take the place of the dorsal lip (fig. 17, *d.l.*). There is, however, no true dorsal lip. The ventral lip is rather large (figs. 16, 17, *v.l.*), straight, with a broad, slightly convex, anterior margin. From this anterior thick ridge projects a thin membrane terminating in a broad rim with granular, refractile cells to which rows of long cilia are attached (figs. 16, 17, *c.r.*). The shape and structure of the ventral lip is unique in this species. Nothing quite the same is known in any other *Fritillaria*.

Fig. 16. View of right side of trunk. × 320. **Fig. 17.** Ventral view of trunk. × 320. **Fig. 18.** Dorsal view of the digestive tract. × 320. **Fig. 19.** Dorsal view of the tail. × 160. **Fig. 20.** Dorsal view of anterior portion of trunk. × 320. Abbreviations: *br.*, branchial opening; *bs.*, blind sac of the intestine; *c.r.*, ring of cells; *d.l.*, dorsal lip; *end.*, endostyle; *h.*, heart; *hd.*, hood; *h.s.*, house; *int.*, intestine; *n.c.*, nerve cells; *oes.*, oesophagus; *oik.*, oikoplast cells; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *r.l.*, ridge of the lip; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.



Figs. 16–20. *Fritillaria lohmanni* sp. nov. (For explanation see p. 424.)

The endostyle is large, its anterior end being considerably wider and its tapering toward the posterior end gradual and pronounced (figs. 16, 17, *end.*). The unusually large, circular branchial openings are distinctly, though not widely, removed from the endostyle. Their median walls are in contact (fig. 17, *br.*). The large hood (figs. 16, 17, *hd.*) gives the anterior portion of the body an angular appearance. The oesophagus is broad and short (fig. 17, *oes.*). The otocyst is conspicuous (fig. 20, *ot.*).

The stomach is thick-walled, elongated, occupying an anterodorsal position. The large, elongated rectum (figs. 16, 17, *rct.*) lies obliquely across the stomach on the ventral side and is connected with the former by an intestine. The latter is surrounded by three accessory blind sacs (figs. 16–18, *bs.*).

The ovary is immediately posteriad of the digestive organs and partly overlaps them. The testis appears as a small globular mass separated from the ovary, posteriad of it, in immature specimens (fig. 17). In other specimens the testis is much larger and high-pyramidal in form (fig. 16, *t.*).

The tail is broad with the proximal end broadest. It tapers gradually to an obtusely rounded point bearing short cilia. The musculature is very wide. The chord is of a uniform width along its entire length (fig. 19, *ms.*, *nch.*).

The length of the body is about 0.37 mm. The width (measuring across the stomach) is 0.16 of its length. The length of the tail is more than 1.5 times the length of the body. Its width is about 0.2 of the length. The width of the musculature is more than 0.5 of the entire width of the tail.

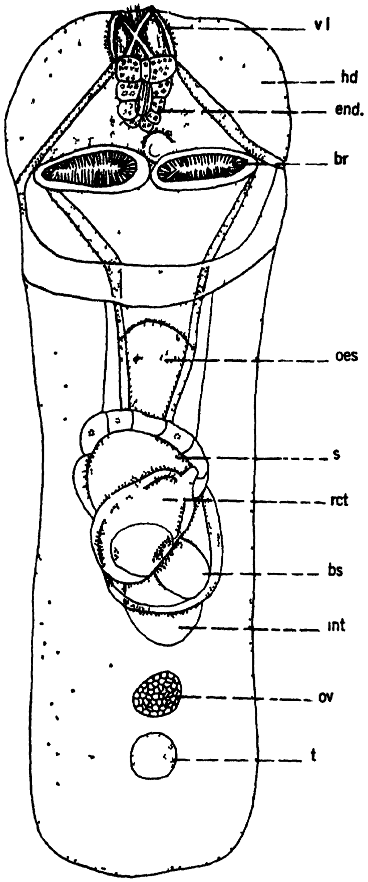
Fritillaria lohmanni is nearest related to *Fritillaria gracilis*, as far as the peculiar, thick ridges around the mouth are concerned. In addition to the thick ridges of *gracilis*, the broad single ventral lip with long cilia has come into existence. The presence of the ventral lip, however, is not the only characteristic that distinguishes it from *F. gracilis*. The large endostyle, the very large branchial openings, the very broad musculature of the tail, and various minor differences separate it distinctly from the latter species.

The species is not abundant here, but on account of its small size it has, perhaps, escaped my notice on some occasions. Two specimens of *Fritillaria lohmanni* were found in the hauls at the pier, one in November, 1921, at a temperature of 14°82; the other in February, 1922, at a temperature of 13°68.

Fritillaria amygdala sp. nov.

Diagnostic characters.—A small species; trunk uniform in width and angular in shape; endostyle broad; branchial openings very much elongated transversely; ciliated fossa conspicuous; oesophagus short; tail wide with broad musculature; hood large.

The trunk of *Fritillaria amygdala* is sac-shaped and uniform in width. The base of the anterior portion of the trunk is slightly convex, bordered by a broad, plasmatic ridge that turns up suddenly at both sides almost at right angles to the base and then extends almost



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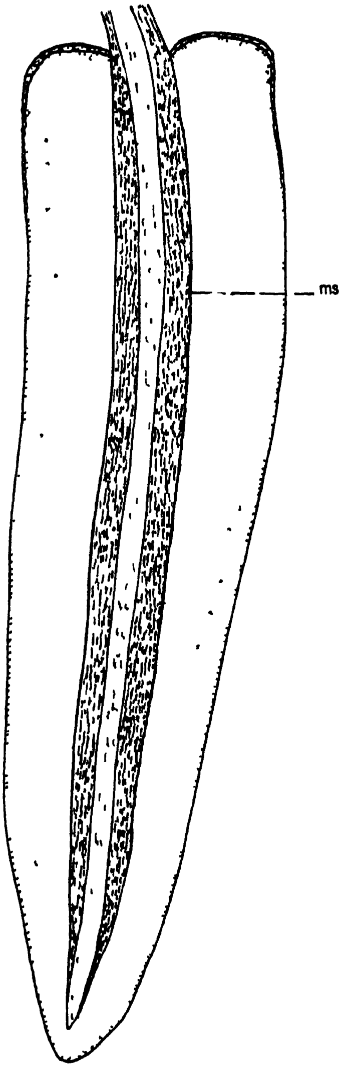

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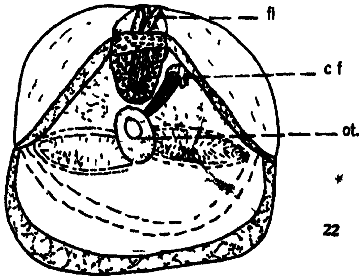

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Figs. 21–23. *Fritillaria amygdala* sp. nov.

Fig. 21. Ventral view of trunk. × 320. Fig. 22. Dorsal view of anterior portion of trunk. × 320. Fig. 23. Dorsal view of tail. × 160. Abbreviations: *br.*, branchial openings; *bs.*, blind sacs of intestine; *c.f.*, ciliated fossa; *end.*, endostyle; *fl.*, flagella; *hd.*, hood; *int.*, intestine; *ms.*, musculature of tail; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

diagonally, both ends meeting at the mouth, making the anterior portion appear triangular. The large hood surrounding the anterior end of the trunk gives it, however, a broad, rounded outline (fig. 21, *hd.*).

The mouth is bordered ventrally by two thick-edged lips, overlapping in the center, and coming to a pointed tip (fig. 21, *v.l.*). No dorsal lip was seen in the two specimens examined. On the dorsal side extend two long whip-like organs bearing strong cilia at their tips (fig. 22, *fl.*). The endostyle is wide, consisting of a few large cells. About the middle of the anterior region in its widest part, are the two strongly elongated, almond-shaped branchial openings placed transversely, occupying the entire width of the body with their median walls adjacent (fig. 21, *br.*). On the dorsal side is a large otocyst overlying the median ends of both branchial openings (fig. 22, *ot.*). The ciliated fossa (olfactory organ) on the right side is conspicuous (fig. 22, *c.f.*).

The short, wide oesophagus leads into a large stomach. The walls of the latter contain large cells (fig. 21, *s.*). From it an intestine leads to the pear-shaped rectum lying obliquely across the posterior part of the stomach on its ventral side (fig. 21, *rct.*). Two large accessory bodies or blind sacs lie one on each side of the intestine (fig. 21, *bs.*).

The reproductive glands consist of two small unconnected globular masses in the sexually immature condition (fig. 21, *ov.*, *t.*), but they may attain a larger size when in mature stage.

The tail is broad, tapering gradually to a narrow, obtusely rounded point (fig. 23). The anterior margin of the tail is slightly emarginate and is considerably thicker than the rest. The tail musculature is of medium width (fig. 23, *ms.*).

Fritillaria amygdala is a small species. The length of the trunk is about 0.34 mm. The width is about 0.33 of its length, measuring from side to side. The species is very much compressed dorsoventrally so that it is almost impossible to get a good side view of it. The length of the tail is 1.67 the length of the trunk. The width of the musculature is about 2 times the width of the chord.

Only three specimens have been found thus far, all in the surface hauls at the pier. One was found on November 26, 1921, temperature 15°15, the other two on December 2, 1921, temperature 14°98.

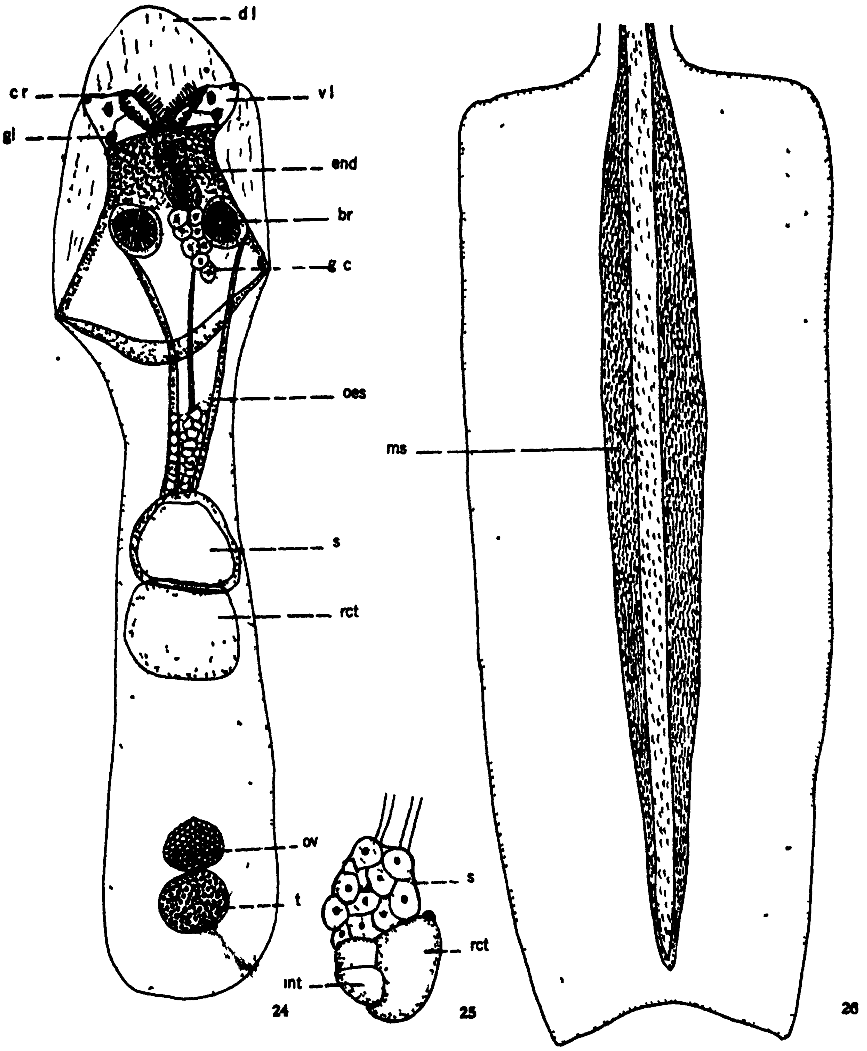
Fritillaria venusta Lohmann

Diagnostic characters.—Trunk slender, its anterior division wider than the posterior; hood large, endostyle accompanied posteriad by a group of cells; branchial openings small and circular; tail wide, truncate-emarginate at end, with wide musculature.

The mouth is provided dorsally with a broad semicircular lip (fig. 24, *d.l.*) and with two smaller lips ventrally (*v.l.*). The anterior margins of the lips are slightly concave and the central sloping edges bear numerous cells with an abundance of cilia. The mouth is also provided with a ring of ciliated cells (fig. 24, *c.r.*).

· The endostyle is slender and nearly equally broad at the two ends. From its posterior end, in the pharynx, there projects a group of conspicuous cells (fig. 24, *g.c.*). The branchial openings are small, circular, and are located near the middle of the anterior portion of the body at the base of the endostyle (fig. 24, *br.*). The oesophagus is short and thick-walled.

The stomach is regular and in a straight line (figs. 24, 25, *s.*). It is almost spherical and its walls are made up of a few large cells. It is followed by the rectum (fig. 24, *rct.*). A narrow, straight intestine on the dorsal side connects the two (fig. 25, *int.*).



Figs. 24–26. *Fritillaria venusta* Lohmann. × 160

Fig. 24. Ventral view of trunk. Fig. 25 View of right side of digestive tract. Fig. 26. Dorsal view of tail. Abbreviations: *br.*, branchial openings; *g.c.*, group of cells; *c.r.*, ring of cells; *d.l.*, dorsal lip; *gl.*, nerve ganglia; *int.*, intestine; *m.s.*, musculature; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

The ovary and testis are small globular masses (fig. 24, *ov.*, *t.*) far removed from the digestive system when immature. At maturity, however, they may assume a different shape.

The tail is wide, the margins nearly parallel. It is broadly but shallowly bifurcated, with sharp angles. The musculature is broad relative to the width of the tail (fig. 26, *ms.*).

The length of the body is about 0.8 mm. Its width is about twice its length. The length of the tail is 1.33 times that of the trunk. The musculature of the tail is about 0.17 of the entire width of the tail and about twice the width of the notochord.

The species agrees very well with the illustrations and description of Lohmann's *Fritillaria venusta,* and is undoubtedly to be considered as the same species. The chief difference lies in the reproductive glands. In Lohmann's illustrations these are shown closely crowded together immediately following the digestive system. In our specimens they were far removed from the stomach and consisted of two globular masses attached to each other.

Only a few specimens have been found in the surface hauls at the pier in December, 1921, and January, 1922, the average temperature being 15°2.

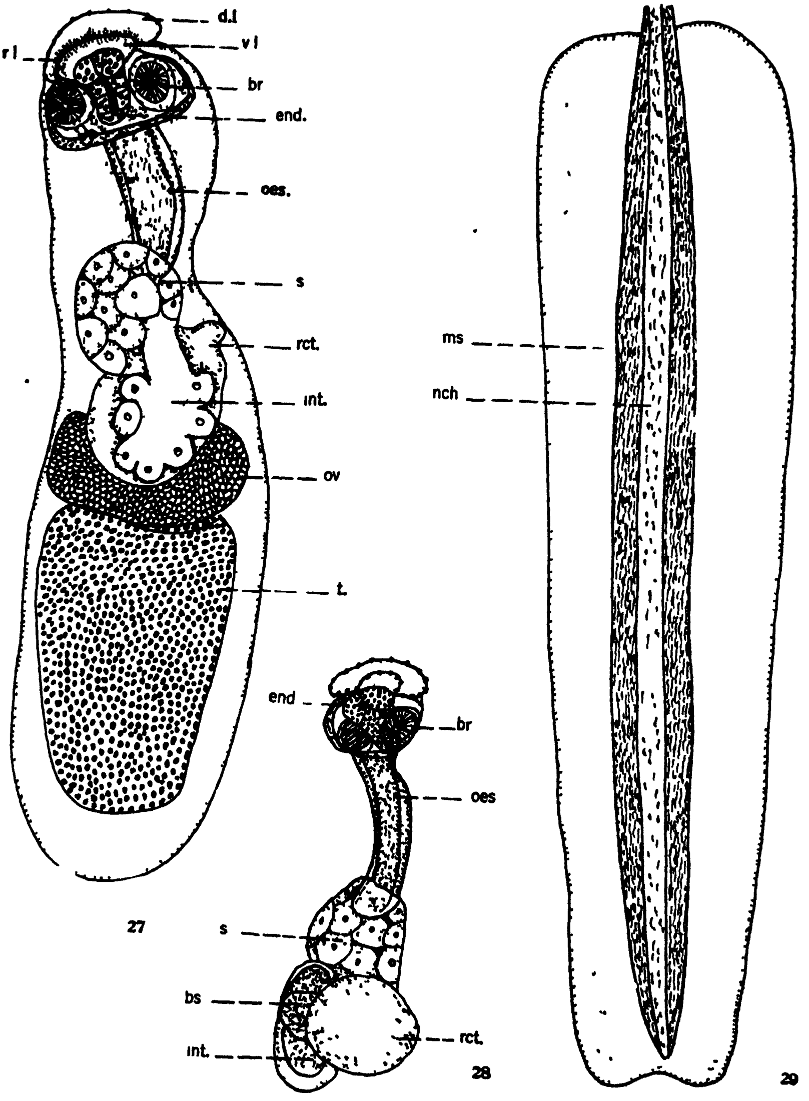
Further occurrence of *Fritillaria venusta* has been reported (Lohmann, 1896) from Cape Verde, from the equatorial stream, from the Guinea-stream, and from the cold island. It was found in depths of 0–650 m. at a surface temperature of 23°2–26°8, and salinity of 34.8–36.4 per cent.

Fritillaria exilis sp. nov.

Diagnostic characters.—A medium-sized species; trunk slender; anterior division of trunk unusually short allowing barely room for the branchial openings and endostyle; branchial openings located far forward on each side of the endostyle and near the mouth; stomach and rectum situated in nearly a direct line in the axis of the trunk, both overlapped by the intestine on the dorsal side; ovary sausage-shaped; testis long; tail long, bifurcated, with a proportionately wide musculature.

The mouth is surrounded by a broad, short ventral lip and by a larger dorsal one (fig. 27, *v.l.*, *d.l.*). The thick margins of both lips are provided at regular intervals with small refractile cells, evidently connecting with cilia. The endostyle is large, being especially broad anteriorly. Alongside the endostyle and well toward the mouth, are the two large, circular, branchial openings occupying all of the free anterior division of the trunk. A narrow transverse plasmatic ridge marks the base of this portion of the trunk on a level with the base of the endostyle and the posterior edges of the spiracles (figs. 27, 28). The oesophagus is long, and strongly ciliated. It begins almost immediately posteriad of the branchial openings (figs. 27, 28, *oes.*).

The oblong stomach is anteriad of the rectum and inclines slightly to the left side. The intestine issues from the dorsal right side of the stomach at about its middle and extends across the rectum (fig. 27, 28. *int.*). Its anterior end is narrow and smooth, but it becomes wider and irregular in its course posteriad on account of the large cells contained in its walls on both sides. The cells are evidently glandular in their nature. The large pear-shaped rectum lies posteriad of the stomach and has a conspicuous nipple-shaped anus. The stomach and the rectum seen from the ventral view appear to be adjacent, hardly touching each other.



Figs. 27–29. *Fritillaria exilis* sp. nov. × 160.

Fig. 27. Dorsal view of trunk. Fig. 28. View of right side of anterior portion of trunk. Fig. 29. Dorsal view of tail. Abbreviations: *br*, branchial openings; *bs.*, blind sacs; *d.l.*, dorsal lip; *end.*, endostyle; *hs.*, house; *int.*, intestine; *ms.*, musculature of tail; *nch.*, notochord; *oes.*, oesophagus; *ov.*, ovary; *r.l.*, cellular ridge of the lips; *rct*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

The ovary is elongated transversely, somewhat sausage-shaped, partly enveloping the digestive system posteriorly. The testis has the form of a truncated cone, more than twice the size of the ovary (fig. 27, *ov.*, *t.*).

The tail is long, rounded at the base with a slight indentation at the tip. The musculature is wide. The chorda is wide and nearly uniform throughout its length (fig. 29).

Fritillaria exilis is easily distinguished from all other species of *Fritillaria* by the unusually short branchial region of its trunk. The branchial openings appear to be practically crowded on both sides of the endostyle while in most other species they are situated posterior to the endostyle. For such a short pharyngeal region the oesophagus is comparatively long.

The trunk is very much compressed dorsoventrally, so that it is difficult to place the animal on its side so as to obtain a side view. The length of the body is 0.65 mm. The width is about 0.2 its length, measuring from side to side in its widest portion. The length of the tail is 1.33 times that of the body.

The species occurs in this region at times in considerable numbers, and then it may disappear completely. It has been found on the following dates:

Fritillaria exilis

Date	Temperature	Number
March 22, 1919	13°4	8
March 24, 1919	14°5	5
April 5, 1919	14°0	1
February 7, 1920	14°42	3
February 9, 1920	13°96	4
March 19, 1920	14°17	1
December 20, 1921	13°92	4

Fritillaria trigonis sp. nov.

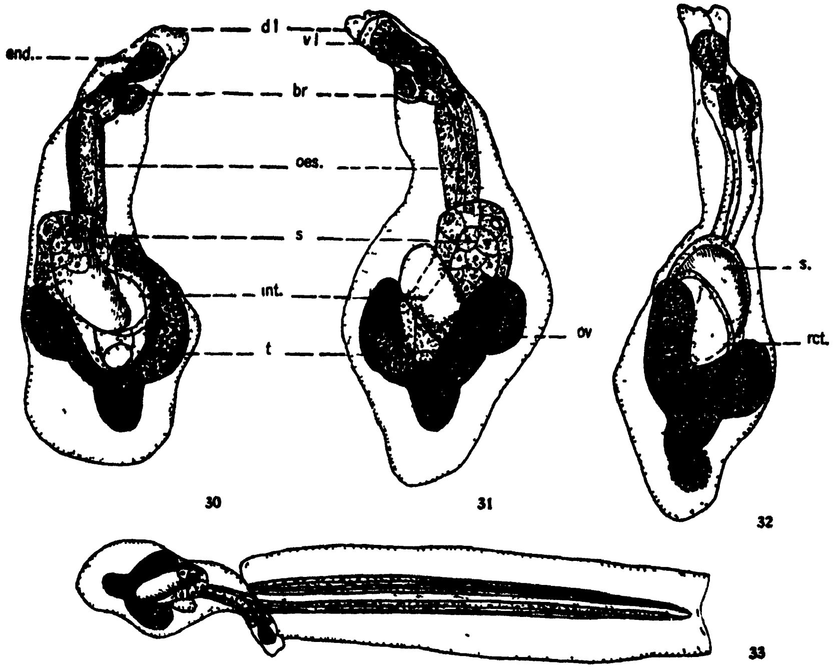
Diagnostic characters.—Trunk short, stout, and irregularly triangular in optical outline; branchial openings circular; reproductive glands crowded around the stomach; tail strap-shaped, posterior end truncate or slightly emarginate; the musculature broad in proportion to the width of the tail.

A short ventral lip and a longer dorsal one surround the mouth (fig. 31). The endostyle is large with its anterior end wider than the posterior one (fig. 30, *end.*). The branchial openings are small, circular, and situated close together posterior to the endostyle. The oesophagus is comparatively long and uniform in width.

The stomach is toward the left side of the body, taking an oblique position with the rectum, which is toward the right side. The stomach is elongated and angular. Its wall contains large cells. On the dorsal side lies the narrow smooth intestine (fig. 30, *int.*). The rectum is large, elongated, and lies posteriad to and on the right side of the stomach.

The reproductive organs form a V-shaped mass crowded around the digestive organs. The spherical ovary is at one end of the left limb of the V (figs. 30, 31, *ov.*). The testis composes the rest of the left limb and the whole of the right limb, of the mass.

The tail is long—nearly twice the length of the trunk—and wide, the uniform width being about 0.2 of the length (fig. 33). The musculature is broad in proportion to the width occupying about one-third of the entire width of the tail. The posterior end is truncate or slightly emarginate.



Figs. 30–33. *Fritillaria trigona* sp. nov.

Fig. 30. Dorsal view of trunk. × 160. Fig. 31. Ventral view of trunk. × 160. Fig 32. View of right side of trunk. × 160. Fig. 33. Dorsal view of the whole animal. × 80. Abbreviations: *br.*, branchial openings; *d.l.*, dorsal lip; *end.*, endostyle; *int.*, intestine; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

The body is very short, differing in this respect from most of the slender-bodied species of *Fritillaria.* The anterior portion of the trunk is narrow. The length is about 0.4 mm. The width (measuring in the widest part) is 0.33 of the length. The length of the tail is nearly twice the length of the trunk. Its width is about 0.2 of its length.

The species is rare in this vicinity. Only two specimens were found in the surface hauls at the pier on January 31, 1919, at a temperature of 13°7.

Fritillaria borealis Lohmann (1896)

Diagnostic characters.—Trunk long-drawn, the surrounding wide cuticle giving the posterior end a triangular appearance; hood delicate; mouth bordered by plasmatic masses; endostyle wide and short; branchial openings small and circular, situated on the sides far removed from endostyle; tail bifurcated.

The mouth is bordered by two large, opaque, plasmatic masses, one on each side of the endostyle, between the dorsal and ventral lips (figs. 36, 37, *pl.*). They protrude in sharp peaks anteriad. The ventral lip is divided by a deep notch thus making two transparent lobes. The dorsal lip is single with a thick ridge (fig. 37, *d.l.*) slightly notched in the center. The endostyle is short and broad with the posterior tip slightly bent dorsad. The small, circular, branchial openings are situated on the sides, widely separated from each other and far distant from the endostyle.

The stomach is slightly elongated. Posteriad to the stomach lies the large globular intestine which bends and enters the large rectum. The latter lies immediately posteriad of the stomach (figs. 36, 37).

The spherical ovary is located close behind the digestive organs. The long, narrow testis is adjacent to the ovary and ends bluntly. It is about three times the size of the ovary.

The tail is bifurcated by a broad, round notch. The musculature is narrow in proportion to the width of the tail (fig. 34).

The length of the trunk is 0.67 mm., its width 0.25 of the length.

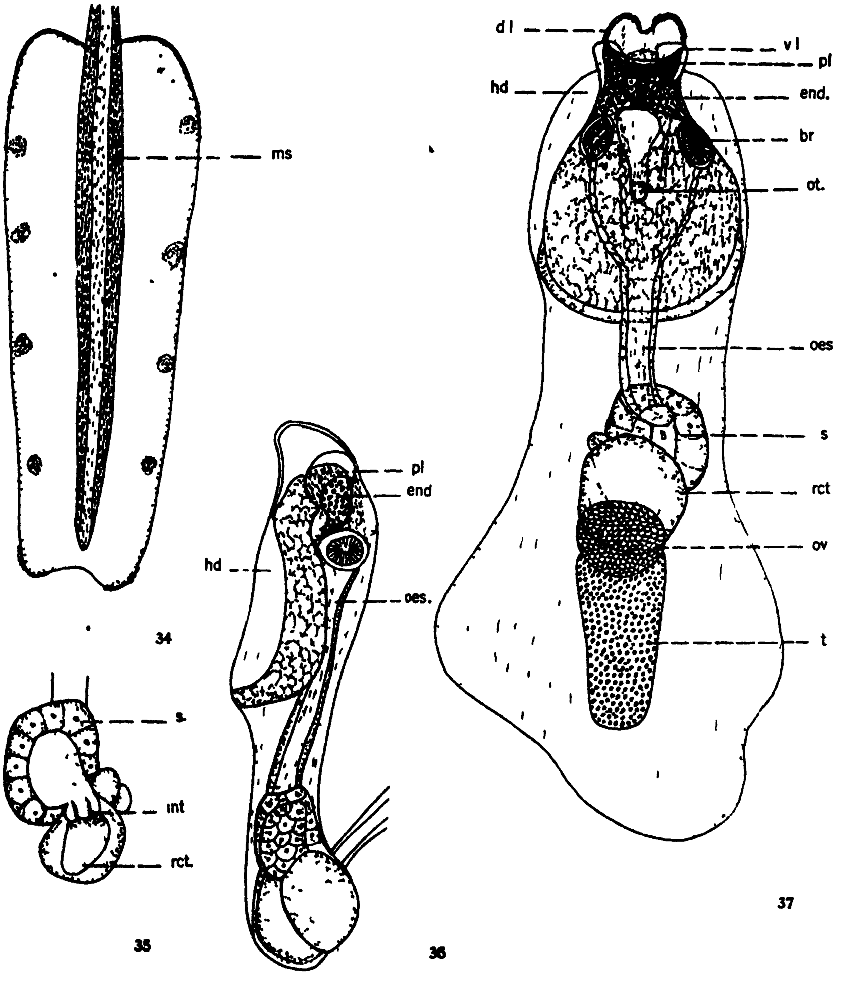
As may be seen from figure 37 our specimens differ slightly in some details from Lohmann's descriptions. One of these differences is in the shape of the dorsal lip. *F. borealis* of Lohmann has a distinctly two-lobed dorsal lip, while in the specimens here described a single non-bifurcated dorsal lip is present. Nevertheless, this Pacific species is so similar to that of the Atlantic in some of the most characteristic features that I do not feel justified in making a separate species but rather assume that it is a variety of Lohmann's *Fritillaria borealis.*

The species occurs occasionally in this region and is found simultaneously with *Fritillaria sargassi.* According to definite records it has been found in the surface hauls at the pier on the following dates:

Fritillaria borealis

Date	Temperature	Number
February 2, 1921	13°76	1
February 3, 1921	11°96	3
February 6, 1921	13°90	1
February 11, 1921	13°86	2
March 18, 1921	13°90	4
March 19, 1921	14°17	1
March 26, 1921	13°45	2
January 26, 1922	13°45	1
January 27, 1922	13°81	3
February 5, 1922	13°59	2

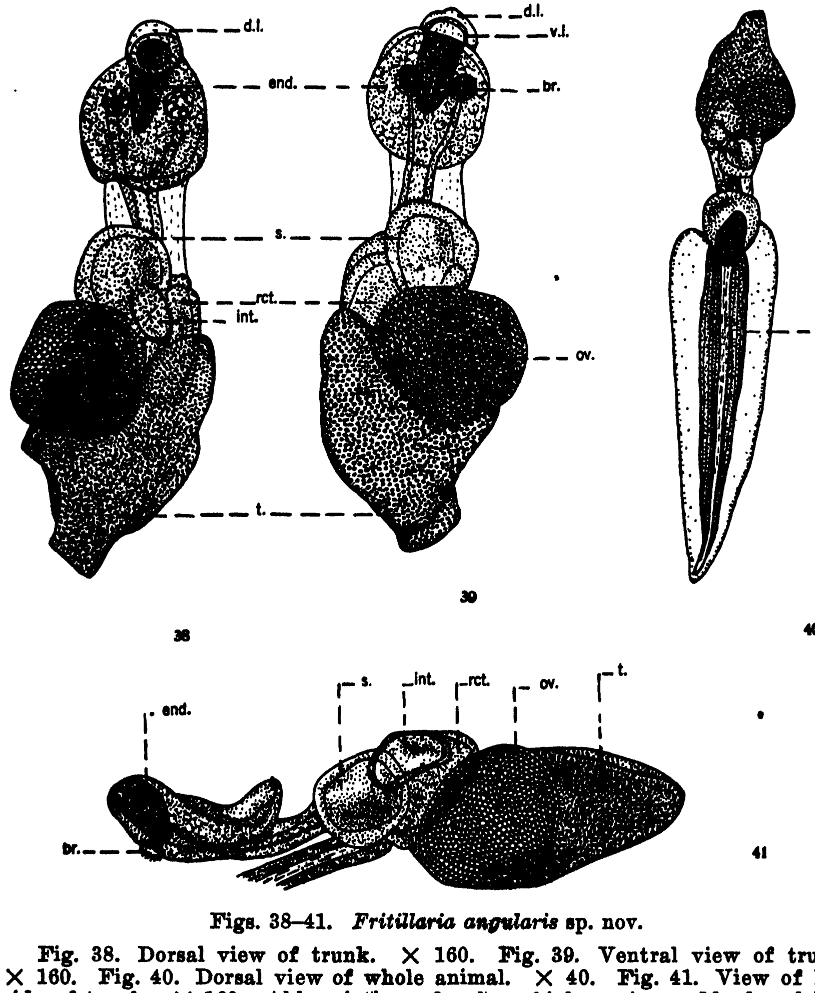
According to the observations of other authors (Lohmann, 1896), *Fritillaria borealis* belongs to the group of cold-water species occurring in great numbers in the Arctic and Antarctic seas. In the Irming Sea and in Davis Straits the species was found during the months of May, June, July, and September at temperatures varying from 1.9–12°3 C. In the Irming Sea it occurred in enormous numbers, 10,000–13,000 individuals per catch. It was found in the Gulf Stream about 60° N. latitude in May at a temperature of 7°1–9° C.; in the Antarctic region, in the Straits of Magellan, in March at a temperature of 4 —6°; near the coast of Finland during October and November.



Figs. 34–37. *Fritillaria borealis* Lohmann.

Fig. 34. Dorsal view of tail. × 80. Fig. 35. Dorsal view of the digestive tract. × 160. Fig. 36. View of right side of trunk. × 160. Fig. 37. Ventral view of trunk. × 160. Abbreviations: *br.*, branchial openings; *d.l.*, dorsal lip; *end.*, endostyle; *hd.*, hood; *int.*, intestine; *oes.*, oseophagus; *o.t.*, otocyst; *ov.*, ovary; *pl.*, plasmatic structure; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

In areas affected by the cold and warm currents the species occurs only during the cold season of the year. In the Baltic Sea it has been found during the months of March and April at a surface temperature of 5°4–8°8 C. In the North Sea it has been found during the months of February, March, April, and November in temperatures of 3.6–12°2 C. Between the current of Labrador and that of Florida it was found in temperatures of 17°2–20°1 C.



Figs. 38–41. *Fritillaria angularis* sp. nov.

Fig. 38. Dorsal view of trunk. × 160. Fig. 39. Ventral view of trunk. × 160. Fig. 40. Dorsal view of whole animal. × 40. Fig. 41. View of left side of trunk. × 160. Abbreviations: *br.*, branchial openings; *d.l.*, dorsal lip; *end.*, endostyle; *int.*, intestine; *ms.*, musculature; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

These data indicate that *Fritillaria borealis* is a highly eurytherm species and is able to endure a variation of temperature of 19° C. Consequently it may be expected to occur in all latitudes, as temperature alone would not bar it from the lower latitudes. The species could easily stand our winter temperature of 13–16° C.

Fritillaria angularis sp. nov.

Diagnositc characters.—Trunk stout, dorsoventrally flattened; endostyle large; branchial openings circular and small, situated alongside the endostyle; testis V-shaped, paired; ovary single, spherical; tail wide, gradually narrowing from base to tip; its musculature wide.

A short semicircular ventral lip and a larger dorsal lip of similar shape surround the mouth (fig. 39). The endostyle is very large (figs. 38–41). The branchial openings are small and circular. They are located on each side of the endostyle (fig. 39, *br.*). The oesophagus is short.

The stomach is round. It is on the left side, anteriad from the rectum. The dorsally situated short intestine leads from the stomach to the broad rectum (figs. 38, 41, *rct.*, *int.*).

The reproductive glands consist of a single large, spherical ovary, located on the left ventral side, partly overlapping the digestive system, and a paired, V-shaped testis which appears very broad and large when seen from either dorsal or ventral surface. It is angular and irregular in form.

The tail is wide and tapers gradually from its base to its pointed distal end. The same is true of the musculature (fig. 40).

The length of the body is less than 0.5 mm. and its width about 0.33 of its length (measuring across the widest part of the body). The length of the tail is about 1.5 times the length of the body. The width of the musculature is about 0.5 of the entire width of the tail.

This species has some resemblance to *Fritillaria sargassi* Lohmann, but it lacks the protruding, long, anterior lobes of *sargassi*. Its tail is different, and the bulky paired testis differs from the slender, single testis of *F. sargassi.*

Only two specimens of the species have been found in the surface hauls at the pier on April 4, 1921, at a surface temperature of 14°9 C.

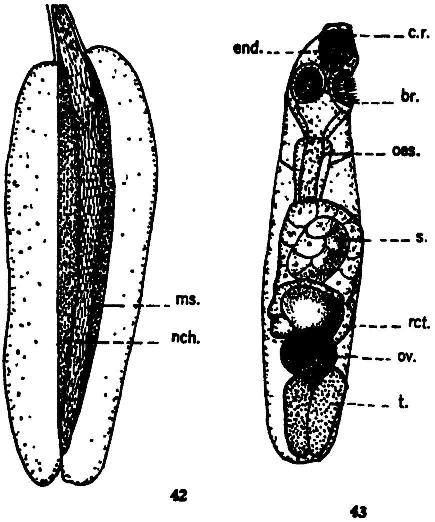
Fritillaria pulchrituda sp. nov.

Diagnostic characters.—Trunk slender, uniform; endostyle short and broad; branchial openings circular; ovary single, spherical; testis paired, elongated; tail bifurcate; hood small.

The trunk of *Fritillaria pulchritruda* is very slender, almost straight and uniform in width gradually becoming narrower toward the anterior end. Traces of the delicate hood were visible in one of the specimens.

The mouth is simple, surrounded by a row of cilia-bearing cells (fig. 43, *c.r.*). The endostyle is broad and short, and slightly curved (fig. 43, *end.*). Immediately posteriad of the endostyle are the circular branchial openings. They are comparatively large occupying the entire width of the trunk (fig. 43, *br.*). The oesophagus is thick-walled and short (fig. 43, *oes.*). The otocyst and ciliated fossa are of the usual type characteristic to the genus.

The digestive system occupies about one-third of the length of the trunk. It is unusually compact and regular, its axis corresponding closely to the axis of the trunk. The stomach is elongated and regular (fig. 43, *s.*). The intestine is short with two large cells, one on each side. The rectum is round.



Figs. 42–43. *Fritillaria pulchrituda* sp. nov.

Fig. 42. Dorsal view of tail. × 80. Fig. 43. Ventral view of trunk. × 160. Abbreviations: *br.*, branchial openings; *c.r.*, ring of cells; *end.*, endostyle; *ms.*, musculature; *nch.*, notochord; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis.

Posteriad of the digestive system, partly overlapping it, is the spherical ovary (fig. 43). The testis consists of two narrow, elongated masses lying side by side posteriad of the ovary.

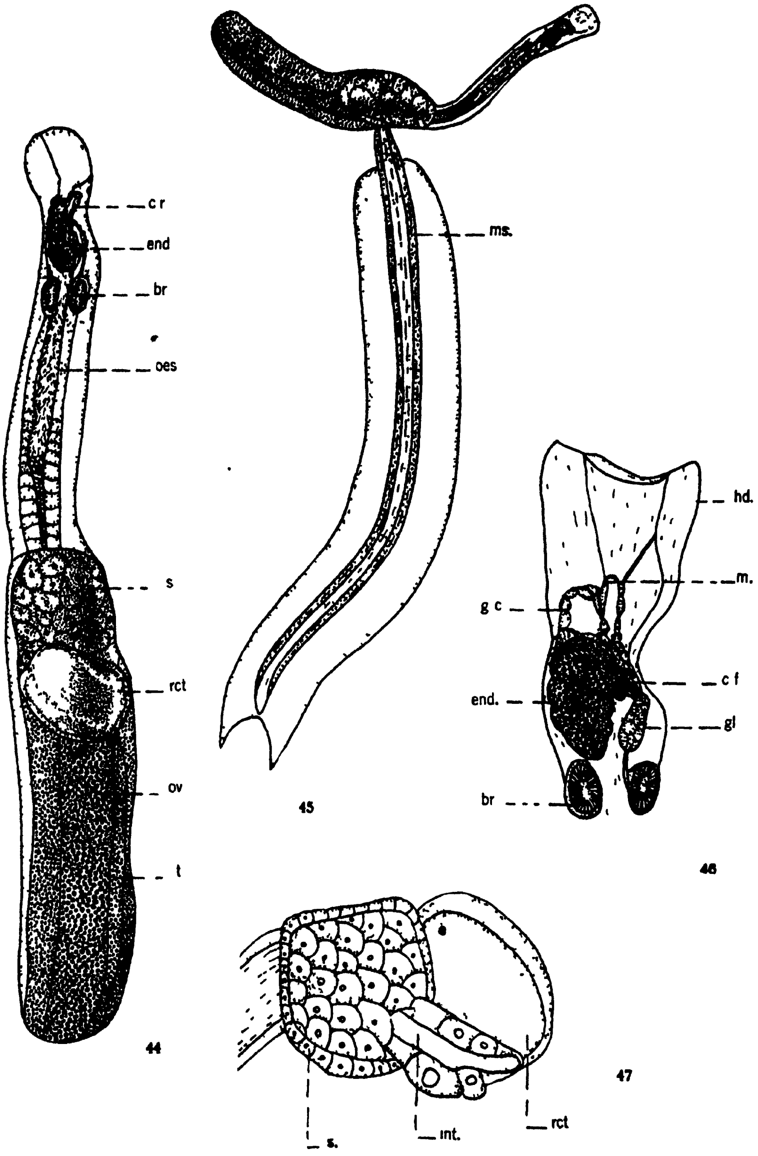
The length of the trunk is only 0.35 mm. Its width is 0.25 of the length. The length of the tail is 1.4 the length of the trunk.

The species is rare in this vicinity. Only 4 specimens have been found, all in the surface hauls at the pier, on February 14, 1920, at a temperature of 14°23 C.

Fritillaria macrotrachela sp. nov.

Diagnostic characters.—A large species. Trunk long and narrow; hood large; mouth simple; endostyle large; branchial openings small, elongated; ovary single, long, surrounded by testis; tail uniform in width, bifurcated by a deep round notch at the end (fig. 45).

Fritillaria macrotrachela is a large species. The body is very slender and almost uniform in width. The trunk is long and narrow. A large hood was present in one of the specimens. The mouth is simple. Around the ridges of the lips is a ring of large ganglion-like cells (fig. 46, *g.c.*). The endostyle is very large and slightly



Figs. 44–47. *Fritillaria macrotrachela* sp. nov.

Fig. 44. Ventral view of trunk. × 80. Fig. 45. The whole animal. × 40. Fig. 46. Dorsal view of the anterior portion of trunk. × 160. Fig. 47. Dorsal view of the alimentary canal. ×160. Abbreviations: *br.*, branchial openings; *c.f.*, ciliated fossa; *c.r.*, ring of cells surrounding the mouth; *end.*, endostyle; *g.c.*, group of cells; *gl.*, ganglia; *hd.*, hood; *int.*, intestine; *m.*, mouth; *oes.*, oseophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis.

curved (fig. 46, *end.*). Posteriad of the endostyle and separated from it are the two small, elongated branchial openings. The oesophagus is long, with thick walls composed of large cells (fig. 44, *oes.*). The ciliated fossa, the otocyst, and the anterior ganglion (fig. 46, *gl.*) show very clearly. They are in the usual positions.

The stomach is large and cuboid in shape, filling the whole width of the trunk. Its walls are made up of large cells. The narrow intestine is accompanied by two pairs of large cells, situated on both sides near the middle of the intestine. The rectum is about half the size of the stomach. It is elliptical in shape situated posteriad of the stomach in an oblique position (fig. 47).

The reproductive glands fill the long posterior portion of the trunk. The single ovary is long and narrow, extending the entire length of the long sac of the reproductive glands. The ovary is surrounded by the testis (fig. 44).

The tail is long and uniform in width from end to end. It is bifurcated by a broad, semicircular notch (fig. 45). The musculature is very narrow, slightly exceeding the width of the chord.

The length of the trunk is about 1.5 mm. Its width is about 0.12 of the length, measuring in the widest part of the trunk across the stomach. The length of the tail is 1.5 times the length of the trunk. Its width is about 0.14 of the length. The width of the musculature is about 0.2 of the entire width of the tail.

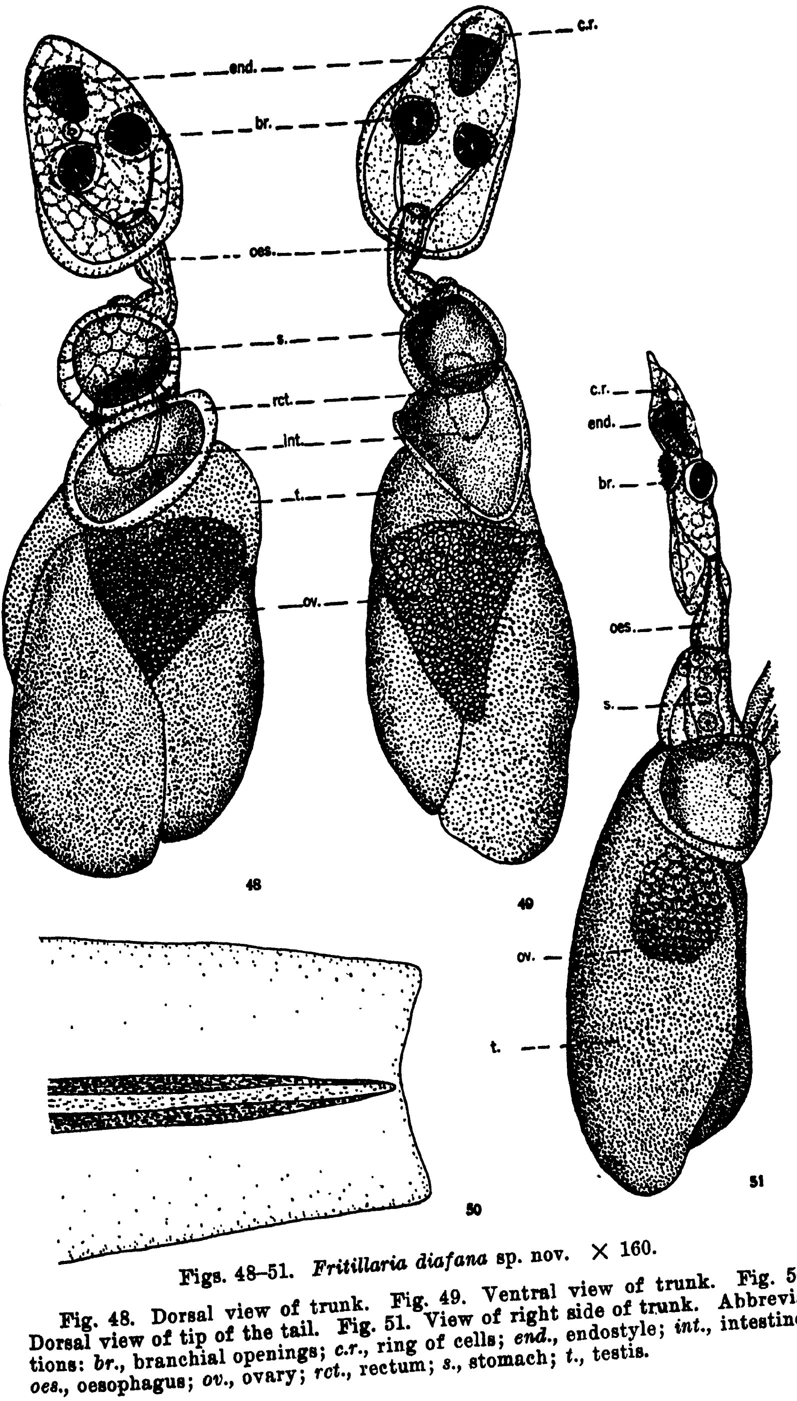
Only two individuals of the species have been found, both in the hauls from the pier. They were not in very good condition. They were found on February 17 and 18, 1920, at a surface temperature of 14°2 C.

Fritillaria diafana sp. nov.

Diagnostic characters.—A large species. Trunk dorsoventrally compressed, angular in shape; mouth simple, surrounded by rows of refractile cells; endostyle short; branchial openings circular, medium sized; stomach ball-shaped; rectum large, oval; reproductive glands bulky; testis and ovary both paired; tail wide, strap-shaped.

Fritillaria diafana is a large species and may be very easily distinguished from any other species of the genus *Fritillaria* by its unusually compressed trunk. The anterior end of the trunk is very narrow when seen from the lateral view, and makes a strong contrast to the broad posterior end (fig. 51). The trunk is broad and irregular in shape, when seen from the dorsal or ventral view (figs. 48–49). The anterior division is almost pear-shaped, with the broad end posteriad. This is followed by a narrower middle portion. The posterior half of the trunk is greatly expanded by the bulky gonads and may reach a width about twice that of the anterior portion, measured from side to side, or in ventral view (fig. 48). The anterior part of the trunk is very transparent.

The mouth is simple without protruding lobes, and surrounded by rows of refractile cells (figs. 49, 51, *c.r.*). The endostyle is broad and short (figs. 48, 49, *end.*). The branchial openings are circular and of medium size. They are well removed from the endostyle and from



Figs. 48–51. *Fritillaria diafana* sp. nov. × 160.

Fig. 48. Dorsal view of trunk. Fig. 49. Ventral view of trunk. Fig. 50. Dorsal view of tip of the tail. Fig. 51. View of right side of trunk. Abbreviations: *br.*, branchial openings; *c.r.*, ring of cells; *end.*, endostyle; *int.*, intestine; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis.

each other. The oesophagus is short and its walls are thick (figs. 48, 49, *oes.*). The heart, the otocyst, and the ciliated fossa are present.

The stomach is spherical with thick walls composed of large cells. It is situated slightly to the left side of the trunk (fig. 48, *s.*). The intestine is short. The elongated rectum is large and is located posteriad of the stomach (fig. 48, *rct.*).

The reproductive glands, when mature, are bulky and constitute the main mass of the trunk. They consist of a pair of huge testes surrounding the paired ovary and may reach a length equal to the rest of the trunk. Their width is twice that of the anterior portion of the body (figs. 48, 49).

The tail is nearly uniform in width throughout. It is nearly truncate at the end, though having a broad, shallow notch. Its musculature is nearly twice the width of the chord (fig. 50). The length of the trunk is 0.75 mm. The width is about 0.14 of its length, measuring across the stomach. The length of the tail is 1.33 times the length of the trunk.

The species is not very common in this region. Only 3 specimens were found as follows: January 9, 1919, temperature 14°5; March 22, 1919, temperature 13°4 C.

Fritillaria haplostoma Fol (1872)

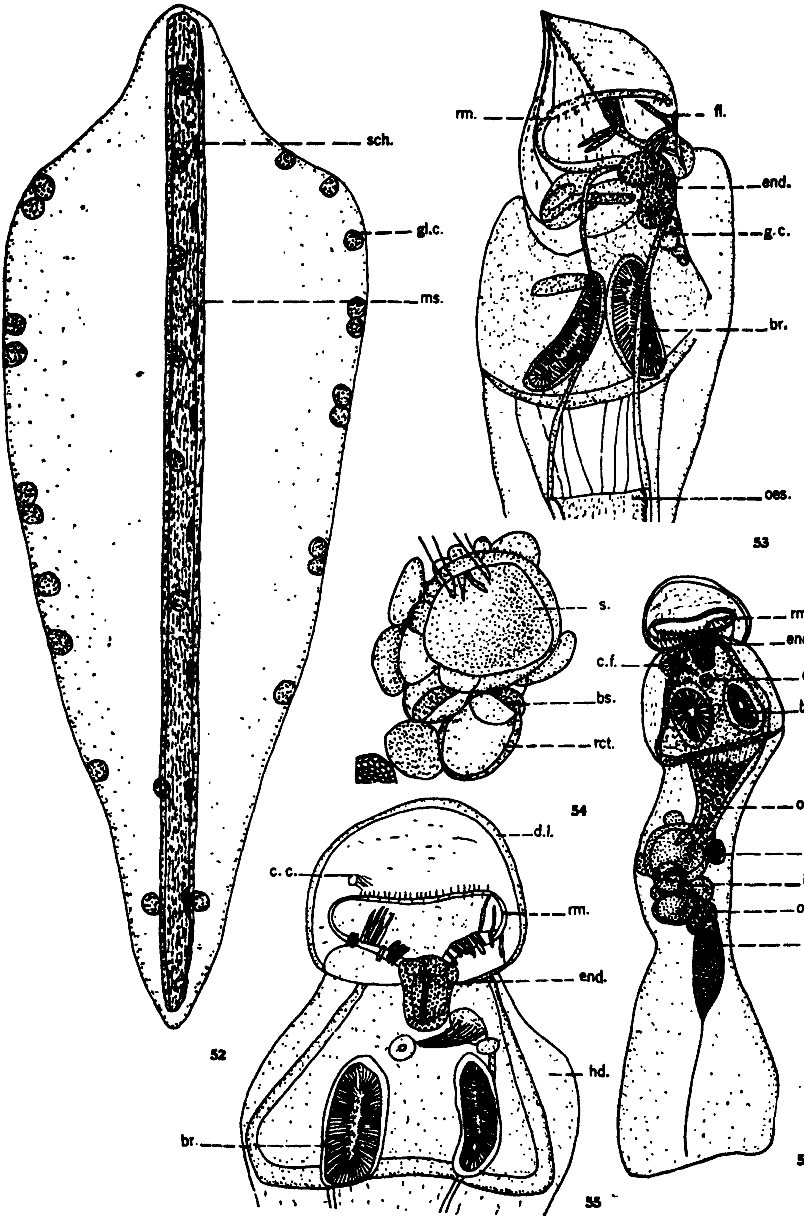
Diagnostic characters.—Trunk slender; dorsal lip large and semicircular; endostyle curved; brancial openings large, elongated; stomach spherical; rectum small; tail wide, triangular, with many glandular marginal cells and a narrow musculature.

The mouth is surrounded by a ring of cilia. The dorsal lip is large, semicircular. On the right side above the ciliated ring is a large cell with cilia (fig. 55, *c.c.*). The endostyle is large, wider at its anterior end, and slightly curved. The ciliated fossa is large (fig. 56, *c.f.*). The branchial openings are large and lenticular. They are situated well posteriad to the endostyle and at a considerable distance from each other (figs. 53, 55, *br.*). The oesophagus is much wider at its anterior end gradually decreasing in width toward its posterior end near the entrance of the stomach (fig. 56, *s.*).

The stomach is spherical with large cells projecting from the surface making the outline uneven (figs. 54, 56, *s.*). The short intestine has two blind sacs, one on each side (fig. 54, *bs.*). The rectum is small about 0.33 the size of the stomach. The spherical ovary is situated immediately posteriad of the digestive system. Posteriad of it extends the elongated testis about 3 times the size of the ovary.

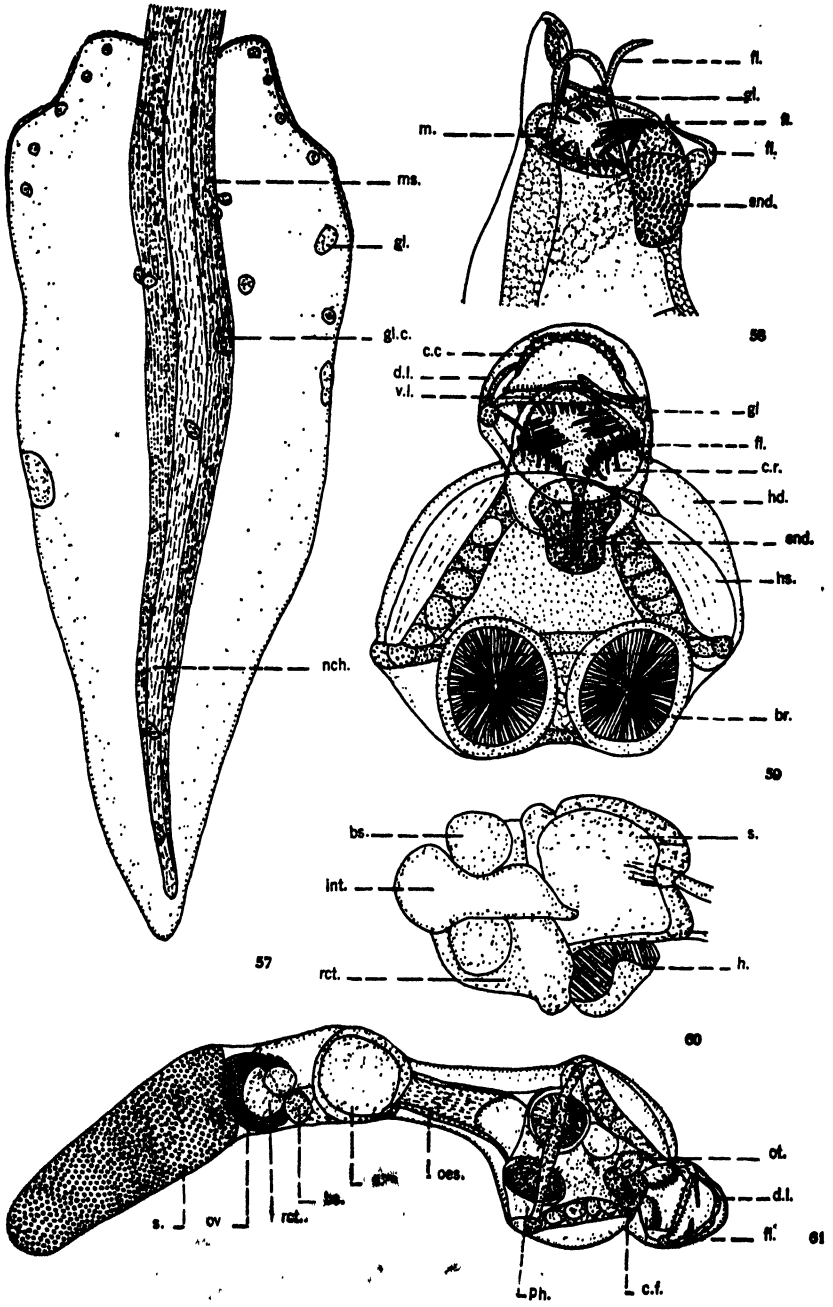
The tail is wide near the proximal end and tapers gradually toward the distal end. The extension of the tail fins anteriorly upon the peduncle is characteristic. The musculature is narrow and the subchordal cells are prominent. Large glandular cells are scattered along both margins of the tail and in the area of the musculature (fig. 52, *gl.c.*).

The tail is slightly longer than the trunk. The width of the tail is about 0.25 of its length. The width of the musculature is almost equal to the width of the chorda.



Figs. 52–56. *Fritillaria haplostoma.*

Fig. 52. Tail. × 160. Fig. 53. Right side of the anterior portion of the trunk. × 160. Fig. 54. View of the left side of alimentary canal. × 160. Fig. 55. Ventral view of anterial portion of trunk. × 160. Fig. 56. Ventral view of trunk. × 80. Abbreviations: *br.*, branchial openings; *b.s.*, blind sacs; *c.c.*, ciliated cells; *c.f.*, ciliated fossa; *g.c.*, group of cells; *d.l.*, dorsal lip; *end.*, endostyle; *fl.*, flagella; *gl.c.*, glandular cells; *hd.*, hood; *int.*, intestine; *ms.*, musculature; *oes.*, oesophagus; *ov.*, ovary; *rm.*, ridge around the mouth; *rct.*, rectum; *s.*, stomach; *sch.*, subchordal cells; *t.*, testis.



Figs. 57–61. *Fritillaria campila* sp. nov.

Fig. 57. Dorsal view of tail. × 160. Fig. 58. View of right side of anterior portion of trunk. × 320. Fig. 59. Ventral view of anterior portion of trunk. × 320. Fig. 60. Dorsal view of the alimentary canal. × 320. Fig. 61. Dorsolateral view of trunk. × 160. Abbreviations: *br.*, branchial openings; *b.s.*,

The length of the trunk is 1 mm. Its width is about 0.25 of the length.

Fritillaria haplostoma never occurs in large numbers but is found occasionally in limited numbers in the surface hauls. It was found on the following dates:

Fritillaria haplostoma

Date	Temperature	Number
January 25, 1920	14°5	1
January 26, 1920	15°0	2
January 30, 1920	14°5	1
February 3, 1920	11°96	2
January 9, 1921	13°96	1

Lohmann (1896) reports the occurrence of *F. haplostoma* from the following places: coast of West Africa, Mediterranean Sea, Sargasso Sea, Florida current, north and south equator currents, and other warm water areas. It was found between the months of August and January in temperatures varying from 9–29°5 C. In some localities it constituted two-thirds of the Fritillarian fauna.

Fritillaria campila sp. nov.

Diagnostic characters.—A small species. Trunk slender and slightly bent; hood delicate; mouth surrounded by complicated system of cilia; endostyle short, broad; branchial openings large, circular; oesophagus short; intestine has two blind sacs; tail wide, gradually tapering toward the distal end, and terminating in a narrow point.

Fritillaria campila is a small species which may be very easily recognized by its very slender trunk with both ends bent ventrad. The anterior portion of the trunk is wider than the posterior three-fourths (fig. 61). A delicate hood is present (fig. 59, *hd.*). The oikoplast epithelium cells are large, and form a triangle. The ciliated fossa and the otocyst are conspicuous (fig. 60, *c.f.*, *ot.*).

The mouth is surrounded by a complex system of cilia and flagella (figs. 58, 59). The dorsal lip is large and semicircular (fig. 59, *d.l.*). A small distance from its edge is a ridge studded with refractile cells and cilia (fig. 59, *c.c.*). The ventral lip is much shorter than the dorsal. Its anterior edge is broad and straight, thickly covered with cilia and flagella (fig. 59, *v.l.*). On each side of the ventral lip is a conspicuous ganglion (fig. 59, *gl.*). From each of these ganglia project two powerful flagella with their tips almost meeting in the center. In the living animals the flagella and cilia are in constant motion beating rhythmically. The mouth is surrounded by thick ridges, from both sides of which project long flagella meeting in the center (figs. 58, 59, 61, *fl.*). A pair of plasmatic pads very much broadened, and

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blind sacs; *c.c.*, ciliated cells; *c.f.*, ciliated fossa; *c.r.*, ring of cells; *d.l.*, dorsal lip; *end.*, endostyle; *fl.*, flagella; *gl.*, nerve ganglia; *gl.c.*, glandular cells; *h.*, heart; *hd.*, hood; *int.*, intestine; *m.*, mouth; *ms.*, musculature; *nch.*, notochord; *ph.*, pharynx; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

forming two leaf-like structures cover the anterior end of the endostyle on its ventral surface (fig. 59, *c.r.*). These are also thickly covered with long flagella, so that the mouth is completely surrounded with cilia and flagella. The endostyle is short and very broad anteriorly, made up of a few cells (fig. 58, 59). The very large circular branchial openings are far posterior from the endostyle (figs. 59, 61, *br.*). The pharynx is large. The short oesophagus is densely lined with cilia (fig. 61, *oes.*).

The stomach is globular, large, and situated in a direct line with the other parts of the digestive system (figs. 60, 61, *s.*). On its dorsal side is a narrow intestine, attached to the dorsal and ventral sides of which are two large blind sacs. The pear-shaped rectum is posterior to the stomach in an oblique position to the latter with the anus pointing anteriad. This is conspicuous and in the usual position in relation to other organs.

The ovary is small and spherical, closely follows the digestive system, and partly overlaps it. Immediately posterior to the ovary is the long, cigar-shaped testis about four times the size of the ovary (fig. 61, *t.*, *ov.*).

The tail is broad with a wide musculature. It tapers gradually toward the distal end (fig. 57). It contains many refractile glandular cells, scattered along the edges of the fins and through the musculature.

The length of the trunk is 0.55 mm. The width is about 0.2 of its length. The length of the tail is 1.5 times that of the trunk. Its width is 0.33 of its length. The width of the musculature is almost 0.25 the width of the chorda.

The species is found from time to time in the surface waters of this region. The following data of distribution are on record:

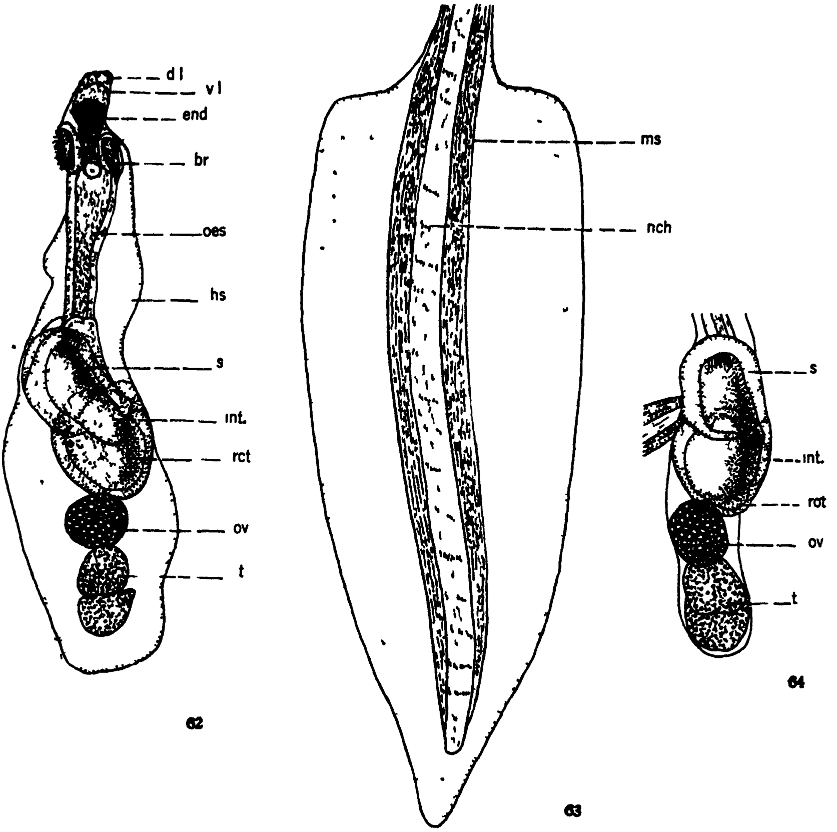
Fritillaria campila

Date	Temperature	Number
November 24, 1919	17°3	2
November 25, 1919	14°5	4
January 13, 1920	14°5	1
January 19, 1920	13°9	3
February 21, 1920	13°4	1
February 22, 1920	13°4	1
February 24, 1920	13°9	3
April 5, 1921	14°4	1
December 4, 1921	15°06	1
December 5, 1921	15°07	2

As far as the observations are recorded, *Fritillaria campila* has occurred in the surface waters of this region at a temperature of 13–15° C., while once it was found on a stormy day at a surface temperature of 17°3 C.

Fritillaria artus sp. nov.

Diagnostic characters.—A small species; trunk slender; mouth simple; endostyle long, curved; branchial openings large, elliptical; pharynx short; oesophagus long; stomach elongated; rectum large; ovary single, testis paired; tail pointed with a narrow musculature.



Figs. 62–64. *Fritillaria artus* sp nov. × 160.

Fig. 62. View of left side of trunk. Fig. 63. Dorsal view of tail. Fig. 64. Dorso-lateral view of the digestive and reproductive systems. Abbreviations: *br.*, branchial openings; *d.l.*, dorsal lip; *end.*, endostyle; *hs.*, house; *int*, intestine; *ms.*, musculature; *nch.*, notochord; *oes.*, oesophague; *ov.*, ovary; *rct*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

The trunk is very slender, almost uniform in width, growing narrower toward its anterior end. The branchial region is unusually short and narrow so that the branchial openings are crowded in on both sides of the endostyle. The widest portion of the trunk is in the region of the digestive system. Traces of the hood seen in one of the specimens.

The mouth is simple, surrounded by a short ventral and a longer dorsal lip (fig. 62, *v.l.*, *d.l.*). No cilia were noticed about the mouth, although the presence of the refractile cells indicates that possibility. The endostyle is long, slender, curved, broad anteriorly, pointed posteriorly (fig. 62, *end.*). Adjacent to its posterior end are the large circular branchial openings (fig. 62, *br.*). The pharyngeal cavity is very short and narrow. The narrow, long, ciliated oesophagus extends from the base of the branchial openings. It is slightly narrower at the posterior than at the anterior end (fig. 62).

The stomach is large and elongated, narrower at the anterior end (figs. 62, 64, *s.*). The large-sized rectum is immediately posteriad of the stomach. The stomach and rectum are connected by an inconspicuous intestine (fig. 64, *rct.*, *int.*). No accessory cells or blind sacs are present. The otocyst is small but distinct.

The single ovary is small and spherical, and lies immediately posterior to and in contact with the digestive system. The testis consists of two regular, round, elongated masses, overlapping each other. These organs are located in the large sac-like cavity of the posterior portion of the trunk (figs. 62, 64, *t.*, *ov.*).

The tail is long-cordate, acuminate at tip, the fins extending forward on the peduncle. Its narrow musculature is strong, especially in the middle region.

The length of the body is about 0.5 mm., the width about 0.2 of its length. The length of the tail is 1.5 times that of the trunk. The width of the tail is 0.33 of its length. The musculature is nearly twice the width of the chorda.

The species is not taken frequently in this region. Only two specimens have been found, in January, 1922, and one of them was in a bad condition.

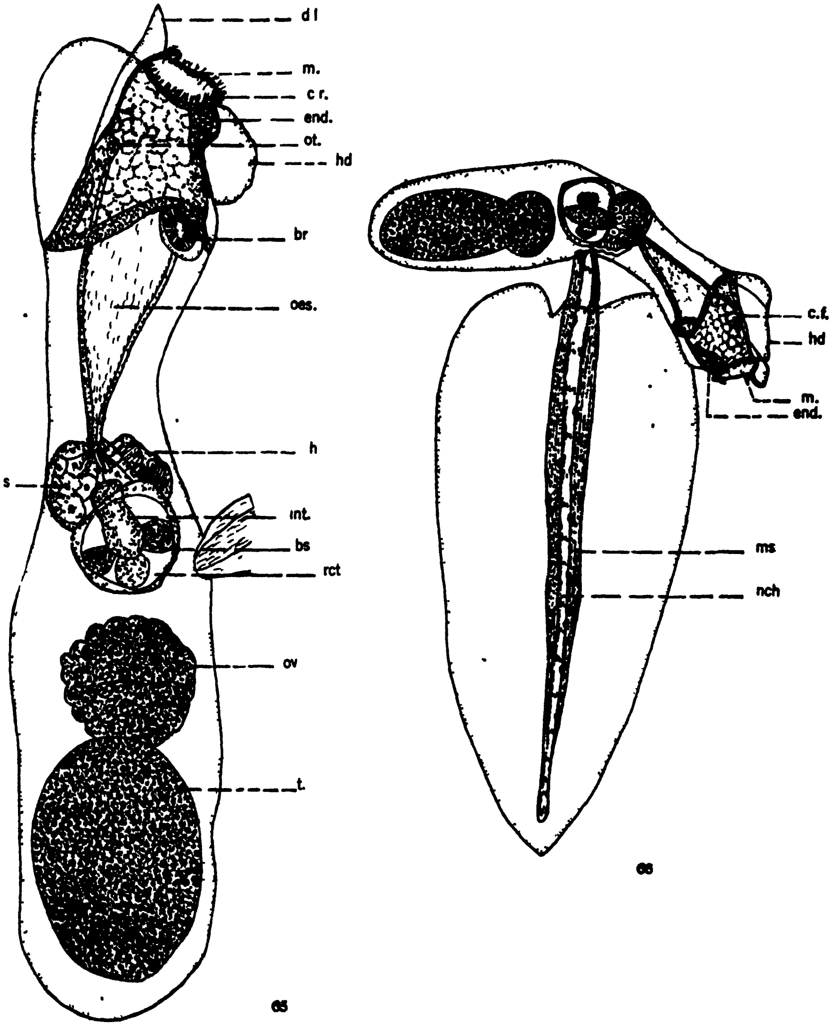
Fritillaria lucibila sp. nov.

Diagnostic characters.—A large species; trunk slender almost uniform in width, slightly constricted in center; mouth simple; endostyle small; branchial openings small and circular, far removed from the endostyle posteriad (fig. 65, *br.*); oesophagus long, its anterior end wide, attenuating posteriad; stomach small, round, situated dorsad; rectum large, located posteroventrad of the stomach; ovary globular; testis oval, twice the size of the ovary; tail cordate, terminating in a broad apex.

Fritillaria lucibila is a comparatively large species, possessing a slender trunk which is very transparent, almost uniform in width, slightly constricted in the center. A medium sized hood extends anteriad. The oikoplast cells extend partly over the anterior portion of the trunk. The pharyngeal cavity is large. The otocyst is conspicuous and the heart is comparatively large, extending along the entire ventral side of the stomach (fig. 65, *h.*).

The mouth (fig. 65, *m.*) is simple, surrounded by a ciliated ring (fig. 65, *c.r.*). The dorsal lip is long (fig. 66, *d.l.*). There is no distinct ventral lip. At the dorsal and ventral corners of the mouth are long tufts of cilia. The endostyle is small and slender, pointed at the posterior end. The branchial openings are small and circular, and are distant from the endostyle posteriad (fig. 65, *br.*). The oesophagus is long and wide in its anterior portion, but becomes very narrow near the stomach, making it funnel-shaped (fig. 65, *oes.*).

The stomach and rectum form a short compact mass. The former is almost spherical, located near the dorsal wall. It is followed and partly overlapped by the nearly spherical rectum, which is situated posteriad and ventrad of the stomach. The two are connected by the narrow intestine (fig. 65, *int.*). The latter is accompanied by three blind sacs, one on each side and one at its posterior end (fig. 65, *bs.*).



Figs. 65–66. *Fritillaria lucibila* sp. nov.

Fig. 65. View of right side of trunk. × 160. Fig. 66. View of right side of the whole animal. × 40. Abbreviations: *br.*, branchial openings; *bs.*, blind sacs; *c.f.*, ciliated fossa; *c.r.*, rings of cells; *d.l.*, dorsal lip; *end.*, endostyle; *h.*, heart; *hd.*, hood; *int.*, intestine; *m.*, mouth; *ms.*, musculature; *nch.*, notochord; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis.

The reproductive glands consist of a spherical ovary detached from the digestive system, and closely posterior to it the elliptical testis. The latter is more than twice the size of the former (fig. 65, *ov.*, *t.*). The tail is wide and cordate attenuating distally to a broad apex. The musculature of the tail is narrow (fig. 66, *ms.*).

The length of the trunk is about 0.85 mm. The width is about 0.16 of its length. The length of the tail is 1.3 times the length of the trunk. The width of the tail is more than 0.33 of its length. The width of the musculature is about twice the width of the chorda.

A single specimen was found on December 19, 1921, at a temperature of 15° C.

Fritillaria limpida sp. nov.

Diagnostic characters.—Trunk sac-shaped, uniform in width as seen from dorsal or ventral side; hood well developed (fig. 67, *hd.*); mouth surrounded by a heavy ridge, and by a dorsal and ventral lip; endostyle slightly curved; branchial openings circular and small; oesophagus short; stomach large and elongated; ovary and testis single, spherical; tail broad, strap-shaped, terminating abruptly in a wide rounded angle; musculature narrow.

Fritillaria limpida is a medium-sized fritillarian. Its sac-shaped trunk is dorsoventrally compressed and slightly bent ventrad (fig. 70). From its ventral or dorsal view the trunk appears slender and uniform in width, with a well developed hood (fig. 67, *hd.*).

The mouth is surrounded by a prominent cellular ridge (figs. 67, 70, *m.*). There is a broad dorsal lip. The ventral lip is equally broad but much shorter than the dorsal (fig. 67, *v.l.*, *d.l.*). The endostyle is large and slightly curved, its posterior tip appearing pointed in side view. The branchial openings are small, circular, and are widely separated from each other (fig. 67). The otocyst is conspicuous (fig. 67, *ot.*). The short oesophagus is thick-walled (fig. 67, *oes.*).

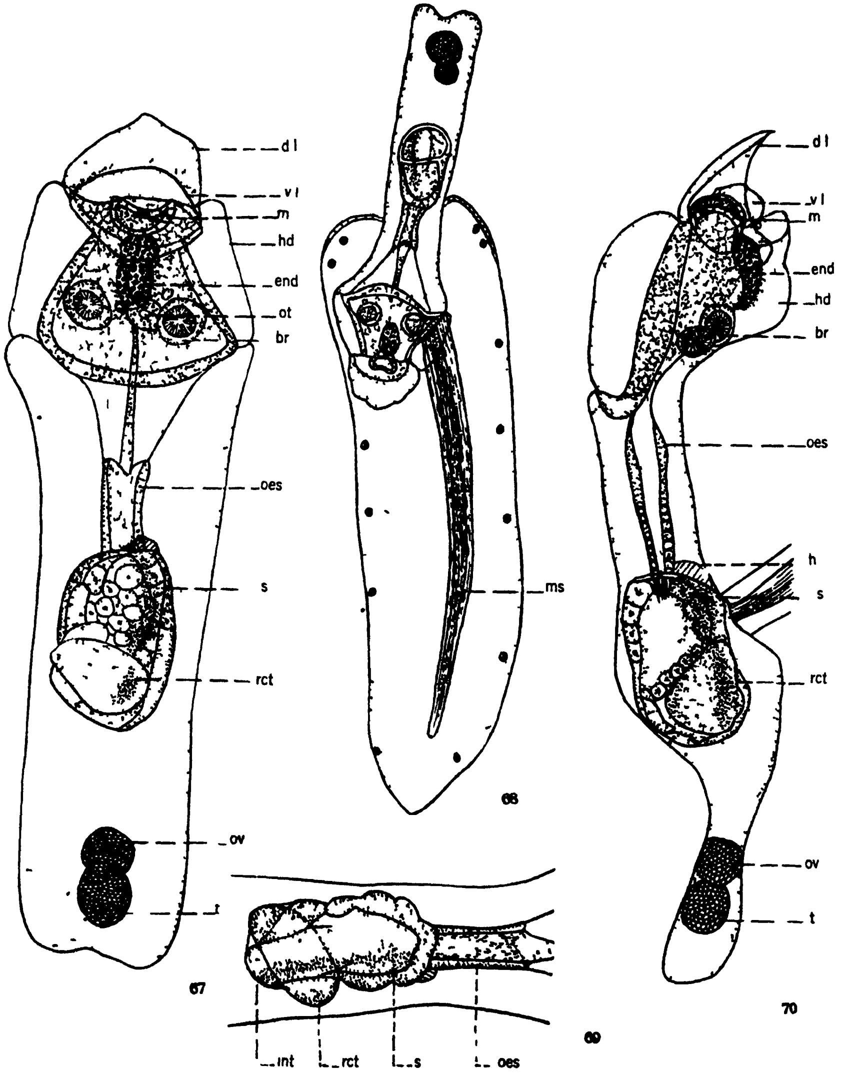
The digestive system forms an elongated, compact mass (figs. 69, 70). The stomach is large and somewhat angular and is covered with many large cells (fig. 67, *s.*). The rectum is pear-shaped (fig. 70, *rct.*) as seen from the ventral side. The intestine is small and inconspicuous without any accessory lobes (fig. 69, *int.*).

The reproductive organs consist (in the few individuals at hand) of two small, globular masses, attached to each other and far removed from the digestive system. Although they appear alike in structure, probably one of them is the ovary and the other the testis. They undoubtedly reach a larger size when in mature state.

The anterior margin of the tail fin is thickened by a row of epithelial cells. The broad, strap-shaped tail is almost uniform in width to near the distal end where it narrows rapidly into wide rounded angle. A number of glandular cells are scattered near both edges of the tail. The musculature of the tail is very narrow, being about 0.12 equal in width to the chorda (fig. 68, *ms.*).

The length of the trunk is about 0.7 mm. The width is about 0.2 the length. The length of the tail is about 1.5 times the length of the trunk.

Fritillaria limpida has a slight resemblance to *Fritillaria venusta.* It is not common in this region, only four individuals having been found, all in the surface hauls from the pier, on January 21 and 22, 1922, at a temperature of 14°5 and 13° C.

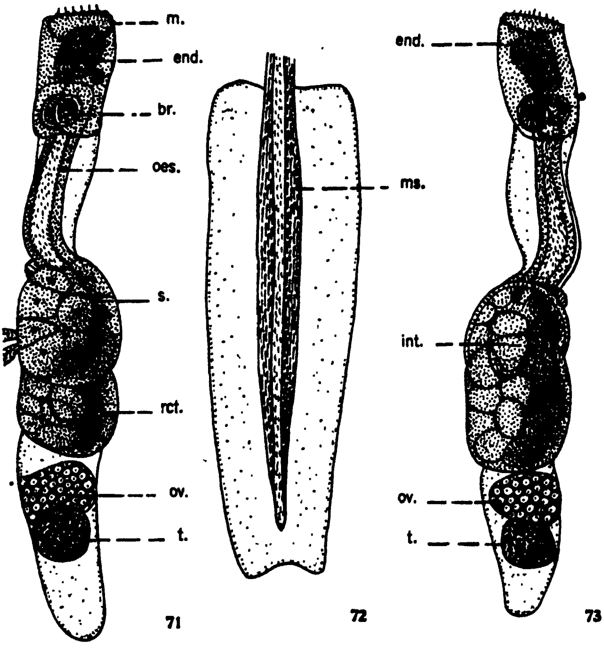


Figs. 67–70. *Fritillaria limpida.*

Fig. 67. Ventral view of trunk. × 160. Fig. 68. Ventral view of whole animal. × 80. Fig. 69. Dorsal view of alimentary canal. × 160. Fig. 70. View of right side of trunk. × 160. Abbreviations: *br.*, branchial openings; *d.l.*, dorsal lip; *end.*, endostyle; *hd.*, hood; *int.*, intestine; *m*, mouth; *ms.*, musculature; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

Fritillaria juncea sp. nov.

Diagnostic characters.—Trunk slender, narrow, uniform in width; mouth simple; endostyle large; branchial openings small, circular, closely adjacent to each other in the median line; oesophagus long; stomach closely anteriad to the large rectum; intestine inconspicuous; ovary and testis globular; tail strap-like with broad musculature, and emarginate posterior end.



Figs. 71–73. *Fritillaria juncea* sp. nov.

Fig. 71. Dorsal view of trunk. × 160. Fig. 72. Dorsal view of tail. × 80. Fig. 73. Ventral view of trunk. × 160. Abbreviations: *br.*, branchial openings; *end*, endostyle; *int.*, intestine; *m.*, mouth; *ms.*, musculature; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis.

Fritillaria juncea is a very slender species, and possesses a trunk which is narrow, uniform in width, and cylindrical at its anterior end. No traces of hood were visible in the few specimens examined. No projecting lips are present. A ring of cilia-bearing cells extends around the simple mouth (figs. 71, *m.*). The endostyle is large and angular. The otocyst is small. The branchial openings are small, circular, and close together in the median line (figs. 71, 73, *br.*). The oesophagus is long, narrow, and thick-walled (fig. 71, *oes.*). The stomach is large, regular, slightly elongated, its walls containing large cells (figs. 71, *s.*). It is directly anteriad to the large rectum, which it overlaps. The smooth, narrow intestine is inconspicuous (figs. 71, 73, *rct.*, *int.*).

The reproductive glands occupy the posterior part of the trunk, distinctly separated from the rectum. They form two round masses. The ovary is semi-spherical. The single testis, closely posterior to the ovary, is also spherical (figs. 71, 73, *ov.*, *t.*).

The tail is of nearly uniform width throughout, and ends with a shallow notch. Its musculature is wide and heaviest in the middle portion (fig. 72, *ms.*).

The length of the trunk is about 0.5 mm. The width is about 0.17 of its length (measuring across the widest portion of the body). The length of the tail is 1.5 the length of the trunk. Its width is about 0.25 of the length. The width of the musculature is about twice the width of the chorda.

The species is rare in this region. Only two specimens were found in the surface hauls at the pier on February 3, 1920, at a temperature of. 13°76 C.

Fritillaria plana sp. nov.

Diagnostic characters.—This is a small species; trunk club-shaped, anterior end of trunk narrow, posterior one broad; mouth simple with a dorsal and ventral lip; endostyle short; branchial openings large, elliptical; ovary and testis long, horseshoe-shaped, surrounding the large rectum and extending posteriad in a prolonged point; tail fin emarginate at both ends, musculature broad.

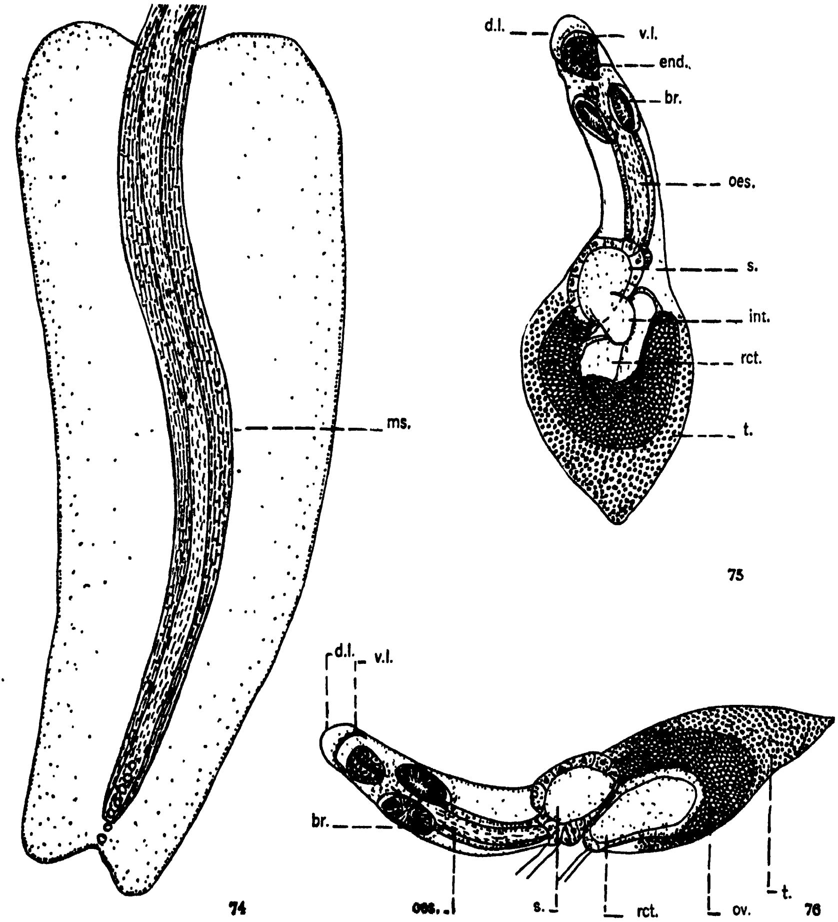
Fritillaria plana may be very easily distinguished by its peculiarly shaped trunk. The trunk with its extremely narrow anterior end and very broad posterior end is somewhat club-shaped (fig. 75). The mouth is simple and has a single ventral lip and a longer dorsal one. The rim of the ventral lip bears a number of refractile cells (fig. 75, 76, *vl.*, *d.l.*). The endostyle is short and broad, almost as wide as long, narrowing toward the posterior end (fig. 75, *end.*). The otocyst is small and inconspicuous. The branchial openings are large, elliptical (figs. 75, 76, *br.*). The oesophagus is long (figs. 75, 76, *oes.*).

The stomach is elongated and pouch-shaped. Its walls are thick, consisting of large cells (figs. 75, 76, *s.*). It lies on the left side, oblique in position so that the oesophagus enters on the right side of its anterior end (fig. 76). On the right side of the stomach is the long rectum with its anterior end lying side by side with the former (fig. 76, *rct.*). The posterior end extends dorsad to the left, and beyond the stomach. The intestine is on the dorsal side having its beginning on the right side of the stomach, leading across the rectum (fig. 75, *int.*).

The genital glands, extending posteriad in a prolonged point, consist of a long, horse-shoe-shaped ovary surrounding the rectum, and a testis surrounding the ovary. When the glands are mature they are large and make the posterior portion of the body bulky so that this end is more than twice the width of the slender anterior end (figs. 75, 76, *t. ov.*).

The tail is long, emarginate at both ends, wider anteriorly and broadly bifurcate at the tip. Its musculature is very broad especially anteriorly (fig. 74, *ms.*).

The length of the trunk is 0.4 mm. The length of the tail is 1.67 times the length of the trunk. The width of the tail is 0.33 its length. The musculature of the tail is wide, about twice the width of the chorda. It diminishes gradually in width toward the distal end, terminating near the end of the chorda.



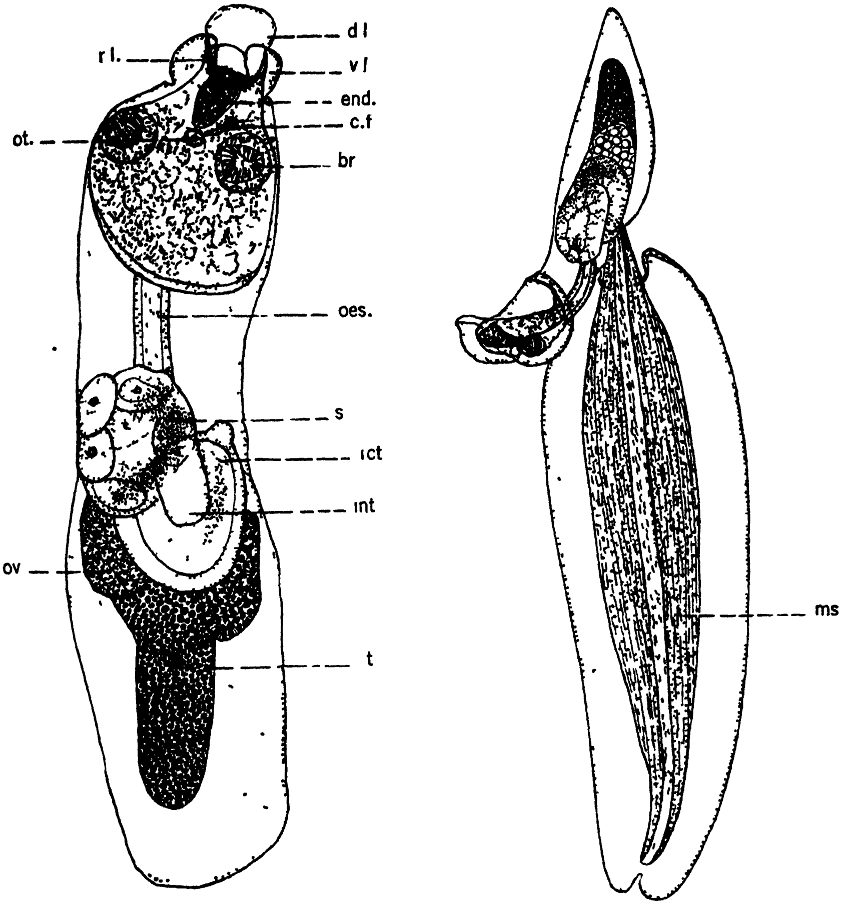
Figs. 74–76. ***Fritillaria plana*** sp. nov. × 160

Fig. 74. Dorsal view of tail. Fig. 75. Dorsal lateral view of trunk. Fig. 76. View of left side of trunk. Abbreviations: *br.*, branchial openings; *d.l.*, dorsal lip; *int.*, intestine; *ms.*, musculature; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

The species is rare in this vicinity. Only a few specimens were found. Four specimens were taken from the surface hauls at the pier on March 9, 11, and 12, 1921, temperature 13°4, 12°3; two were found on December 3 and 4, 1921, temperature 15°6, 14°9.

Fritillaria ritteri Aida (1907)

Diagnostic characters.—Trunk broad and long, dorsoventrally compressed; hood conspicuous; ventral lip separated into two lobes; dorsal lip single, longer than the ventral one; branchial openings small, circular; oesophagus short; genital glands T-shaped; tail narrowly bifurcated at the tip, its musculature wide.



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Figs. 77–78. *Fritillaria ritteri* Aida.

Fig. 77. Dorsal view of trunk. × 160. Fig. 78. The whole animal. × 40. Abbreviations. *br.*, branchial openings; *c.f.*, ciliated fossa; *d.l.*, dorsal lip; *end.*, endostyle; *int.*, intestine; *ms.*, musculature; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *r.l.*, ridge of lip; *s*, stomach; *t.*, testis.

The species *Fritillaria ritteri* was first described by Aida (1907). It is very similar to *Fritillaria sargassi* but differs from it particularly in the absence of the large plasmatic palp-like structures of the mouth of *F. sargassi.* Other differences are found in the shape of the tail and that of the oesophagus.

The mouth is surrounded by a single dorsal lip longer than the ventral one, and a ventral one separated by a deep notch into two lobes (fig. 77, *d.l.*, *v.l.*). A heavy ridge composed of a row of plasmatic cells encircles the mouth (fig. 77, *r.l.*). The ciliated fossa and the otocyst are small but distinct (fig. 77, *c.f.*, *ot.*). The endostyle is thick and slightly bent dorsad. The branchial openings are small and circular and are widely separated from each other, situated near the lateral wall (fig. 77, *br.*). The oesophagus is short, narrow, and straight (fig. 77, *oes.*).

The stomach lies on the left side of the trunk. It is elongated, pouch-shaped. The large rectum is posteriad and on the right side of the stomach. It is pear-shaped with the anus pointing anteriad (fig. 77, *rct.*). The straight narrow intestine is on the dorsal side of the digestive system (fig. 77, *int.*).

The reproductive glands are T-shaped with the stem of the T pointing posteriad. The spherical ovary is on the left side of the rectum. Adjacent to it is the testis extending posteriad and branching off to the right partly surrounding the rectum on the right side (fig. 77, *ov.*, *t.*).

The tail is broad, bifurcated at the end by a deep narrow notch. The anterior end of the caudal fin is separated from the main stem by a deep notch (fig. 78).

The length of the trunk is about 0.6 to 0.8 mm. The width is about 0.2 of the length. The length of the tail is 1.67 times the length of the trunk. Its width is about 0.25 of the length. The musculature is very wide, especially in the middle of the tail where it occupies about 0.5 of the entire width of the tail, and is about 4 times the width of the chorda.

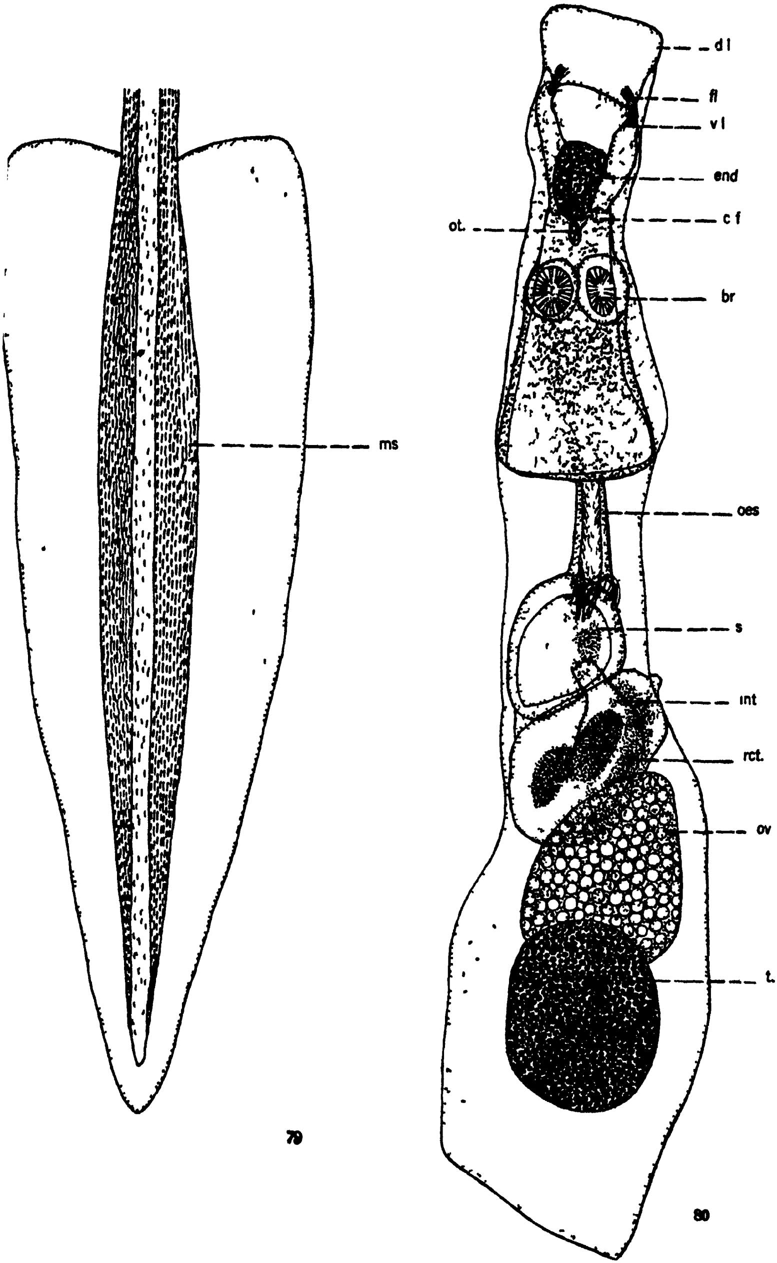
The species occasionally occurs in the surface waters of this region. It has been found in the hauls taken at the pier on the following dates:

Fritillaria ritteri Aida

Date	Temperature	Number
January 15, 1921	14°3	2
January 17, 1921	14°5	1
January 18, 1921	14°5	1
January 30, 1921	14°53	3
January 31, 1921	14°27	1
December 3, 1921	15°06	1
December 4, 1921	14°99	2

Fritillaria ritteri, like all other species of *Fritillaria* appears suddenly and may be found in the surface waters for a few days in succession, and then may not be seen for months or years afterward. Undoubtedly the tunicates are driven to the coast by currents, tides or waves.

Aida (1907) found two specimens of *Fritillaria ritteri* near the western coast of Kinshu, Japan. The date of their occurrence is not given, but probably they were found in summer because he makes the general statement that the Copelata are usually driven to the Japanese coast in the summer when the south wind prevails.



Figs. 79–80. *Fritillaria nitida* sp. nov.

Fig. 79. Dorsal view of tail. × 80. Fig. 80. Dorsal view of trunk. × 160. Abbreviations: *br.*, branchial openings; *c f.*, ciliated fossa; *d.l*, dorsal lip; *fl.*, flagella; *end.*, endostyle; *int.*, intestine; *ms.*, musculature; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; t., testis; *v l.*, ventral lip.

Fritillaria nitida sp. nov.

Diagnostic characters.—This is a large species; trunk long, slender; mouth surrounded by a large dorsal lip and by a shorter ventral one; endostyle broad, short; branchial openings large, touching each other in the median line; stomach pouch-shaped, shifted to the left side of the trunk; rectum large, pear-shaped; ovary elliptical; testis spherical; tail broad, tapering gradually to a narrow point, the anterior end of the fins emarginate.

Fritillaria nitida is a large species, broad, tapering gradually to a narrow point, the anterior end of the fins emarginate.

The trunk of *Fritillaria nitida* is very slender, dorsoventrally flattened, having a long, drawn appearance. The anterior region, including the respiratory and digestive systems, is comparatively longer than in most other species of *Fritillaria.* A hood is present, but it was in a bad condition in the one specimen found so that its shape and size could not be determined. The otocyst and ciliated fossa are small, located in the usual position (fig. 80, *ot.*, *c.f.*). The heart is conspicuous. The oikoplast epithelium is restricted to the ventral side of the branchial region.

The mouth is simple and has a broad dorsal lip with its anterior margin truncate, and a broad but shorter ventral one (fig. 80, *d.l.*, *v.l.*). On each side of the interior surface of the dorsal lip is a large refractile cell from which arises a tuft of long cilia guarding the entrance to the mouth (fig. 80, *fl.*). The endostyle is broad and short (fig. 80, *end.*). The branchial openings are large and circular and are in contact with each other, occupying the entire width of the trunk in that region. They are far posterior from the endostyle (fig. 80, *br.*).

The anterior division of the trunk is long, somewhat pyramidal in shape. The pharynx is large. The oesophagus is short, narrow and straight, entering the stomach on the anterior right side, its long tuft of cilia extending far into the stomach (fig. 80, *oes.*).

The digestive system consists of a small elongated pouch-shaped stomach situated anteriad to the rectum and nearer to the left side of the trunk (fig. 80, *s.*). The large elongated, pear-shaped rectum is adjacent to the stomach posteriad, taking an oblique position in relation to the latter, with its nipple-shaped anus pointing anteriad (fig. 80, *rct.*). The intestine is on the dorsal side. It is elongated and smooth, without any accessory bodies.

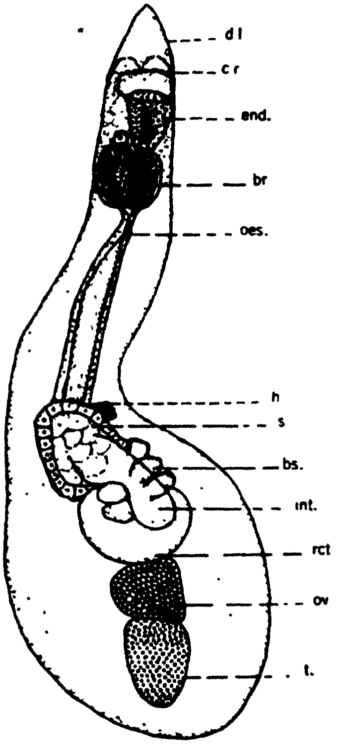
The reproductive glands occupy the posterior portion of the trunk. The elongated, oval-shaped ovary is situated immediately posteriad of the digestive system, partly over-lapping the rectum. The spherical testis is posteriad of and adjacent to the ovary (fig. 80, *ov.*, *t.*).

The tail is broad anteriorly and tapers gradually toward the distal end, the anterior end of the fins emarginate. Its musculature is very wide (fig. 79).

The length of the trunk is about 1.9 mm. Its width is 0.14 of the length. The length of the tail is 1.25 times the length of the trunk. Its width is about 0.2 of its length. The width of the musculature is more than 3 times the width of the chorda. Only one specimen has been found in a surface haul at the pier on January 20, 1921, at a temperature of 13°88 C.

Fritillaria clava sp. nov.

Diagnostic characters.—Trunk club-shaped, its anterior portion very narrow, posterior portion four times the width of the anterior; mouth simple, surrounded by a single dorsal lip and by a pair of short ventral lobes; endostyle short with posterior end turned dorsad; branchial openings large, elliptical, crowded together medianly; stomach slightly to the left side, the rectum to the right; intestine long, with accessory bodies; tail long, bifurcated by a deep notch, anterior end of the fins truncate, but with rounded angles.



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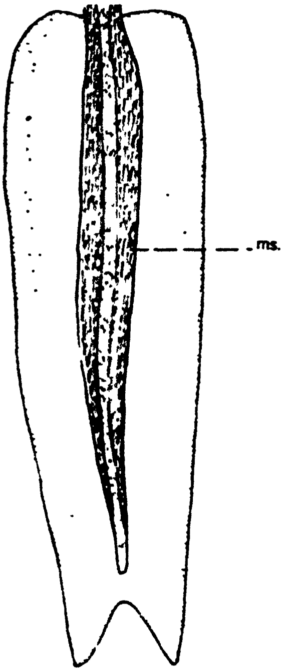

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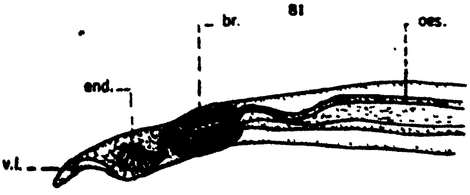

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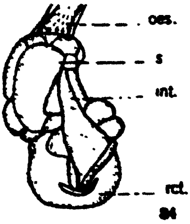

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Figs. 81–84. *Fritillaria clava* sp. nov.

Fig. 81. Dorsal view of trunk. × 160. Fig. 82. Dorsal view of tail. × 80. Fig. 83. View of left side of the anterior portion of trunk. × 160. Fig. 84. Dorsal view of alimentary canal. × 160. Abbreviations: *br.*, branchial opening; *b.s.*, blind sacs; *c.r.*, ring of cells; *d.l.*, dorsal lip; *end.*, endostyle; *h.*, heart; *int.*, intestine; *ms.*, musculature; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

Fritillaria clava is easily identified by its club-shaped trunk (fig. 81). The anterior end as far as the stomach, is narrow and almost uniform in width and appears like a handle attached to the ball-shaped posterior portion of the trunk, which is about 3 times the width of the former. Whether the hood is very delicate or poorly developed, is not known as no traces of its presence were found. The trunk is very transparent. The oikoplast epithelium is restricted to the ventral side anterior to the branchial openings. The otocyst is small.

The mouth is simple surrounded by a ring of refractile cells (fig. 81, *c.r.*). The dorsal lip is long with its upper margin terminating in a rounded point. The very much shorter ventral lip is separated by a deep median notch into two lobes. The endostyle is short and thick, broader at its anterior end, with its narrower posterior end turned up dorsad (figs. 81, 83, *end.*). The large elliptical branchial openings are immediately posterior to the endostyle. They are close together (figs. 81, 83, *br.*). The oesophagus is long and narrow (fig. 81, *oes.*). The elongated stomach lies in a slanting position with its cardiac end turned to the left (fig. 81, *s.*). The rectum on the ventral surface overlaps the posterior portion of the stomach, taking a position in line with the longitudinal axis (figs. 81, 84, *rct.*). The narrow, long intestine is on the dorsal side and is provided on each side with a pair of accessory bodies or blind sacs (fig. 81, *bs.*). The reproductive glands consist of a spherical ovary, located posteriad of the rectum, and an elongated testis posteriad of the ovary (fig. 81, *ov.*, *t.*).

The tail fins are nearly parallel edged, bifurcated by a broad, deep triangular notch at the posterior end, anterior end of the fins truncate but with rounded angles. Its musculature is of medium width occupying about one-fifth of the entire width of the tail and being more than twice the width of the chorda (fig. 82, *ms.*).

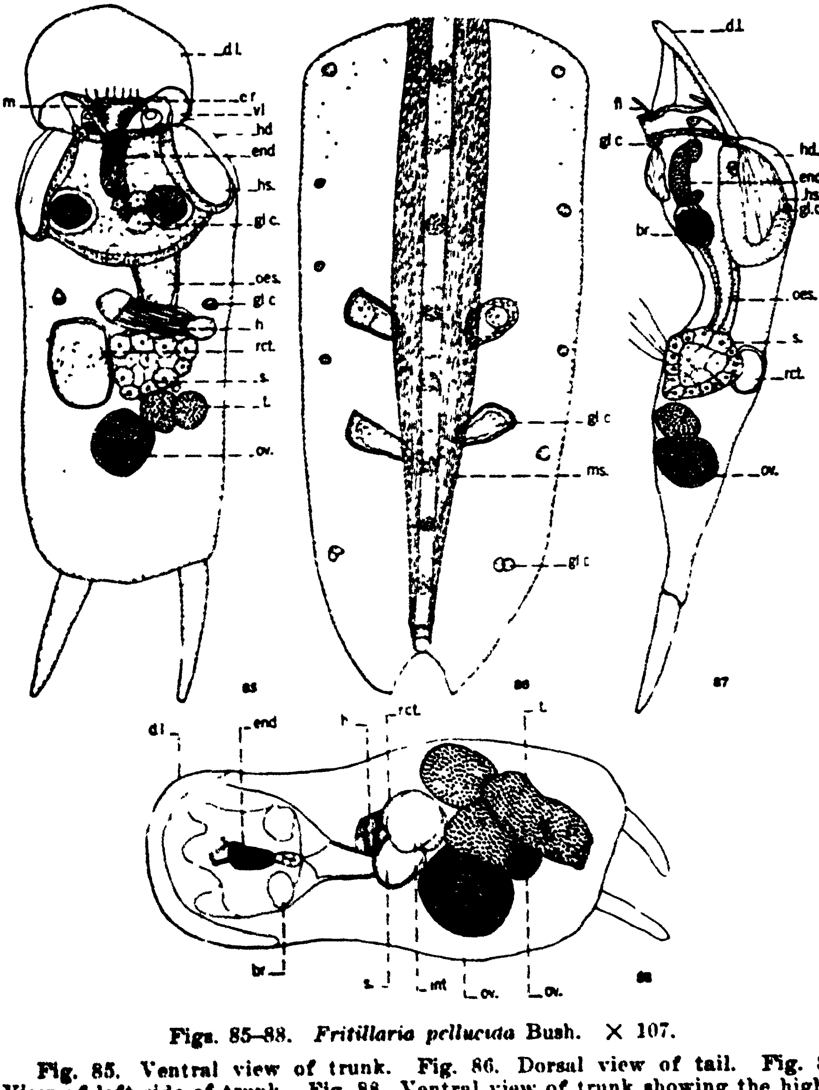
The length of the body is about 0.66 mm. The width is about 0.14 of its length measuring across the anterior end of the stomach. The length of the tail is 1.75 times the length of the trunk.

Only two individuals of the species have been found in the surface waters at the pier on January 4, 1922, at a temperature of 14°.52 C.

Fritillaria pellucida Bush (1851)

Diagnostic characters.—This is a large species; trunk stout, sac-shaped with two long, conical processes at the posterior end; hood prominent; mouth surrounded by rows of refractile cells and cilia; dorsal lip large, round, ventral lip divided by a deep notch into two lobes; endostyle long with both ends turned up dorsad; branchial openings circular medium-sized; a group of large cells at the base of the pharynx; oesophagus short; stomach and rectum in transverse position; intestine short; heart larger than in most species; ovary globular, testis elongated; in young specimens both reproductive glands globular; tail broad, bifurcated with V-shaped notch at the posterior end, especially characterized by two pairs of huge glandular cells.

Fritillaria pellucida is unusual in that it has been well described by various authors (Fol, Lohmann, Aida). It is the only species of *Fritillaria* characterized by a pair of long cone-shaped processes about 0.25 of the length of the trunk, projecting from the posterior end, and two pairs of huge glandular cells on the ventral surface of the tail. Due to these characteristics and to its large size and lively locomotion, this appendicularian rarely escapes notice.



Figs. 85–88. *Fritillaria pellucida* Bush. × 107.

Fig. 85. Ventral view of trunk. Fig. 86. Dorsal view of tail. Fig. 87. View of left side of trunk. Fig. 88. Ventral view of trunk showing the highly developed sexual glands and the shriveled-up condition of the rest of the trunk. Abbreviations: *br.*, branchial openings; *c.r.*, ring of cells; *d.l.*, dorsal lip; *end.*, endostyle; *fl.*, flagella; *gl.c.*, glandular cells; *h.*, heart; *hd.*, hood; *int.*, intestine; *m.*, mouth; *ms.*, musculature; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

The trunk is broad and uniform in width when seen from the ventral side (fig. 85). It is dorsoventrally compressed, appearing narrow and long drawn from the lateral view (fig. 87). The hood is well developed, and the house is usually very small and rudimentary, and is covered by the hood (figs. 85, 87, *hs.*), known as the so-called *Haus Anlage.* The trunk also has a number of epidermal glandular cells located in the outer ectothelial layer. The glands are more numerous in young specimens than in older ones, but usually there are 4 in the genital region, a pair near the anterior end of the endostyle, a pair near the heart, and a group of large glandular cells at the posterior end of the endostyle near the base of the pharynx (fig. 85, *gl. c.*).

The mouth is surrounded by a row of refractile cells bearing cilia (fig. 85, *c.r.*). The dorsal lip is well developed, round, tongue-shaped. The ventral lip is divided by a deep notch into two lobes whose thick edges are covered with a row of tactile cells. The endostyle is long with both ends turned up dorsad. The medium-sized, circular branchial openings are far apart, lying close to the lateral walls near the posterior end of the pharynx (fig. 85, *br.*). A group of large cells lies at the base of the pharynx. The oesophagus is wide and short.

The stomach is short and broad and is located on the left side, while the elongated rectum is on the right side (fig. 85, *s.*, *rct.*). A large intestine almost the size of the rectum lies on the dorsad side connecting the two (fig. 88, *int.*).

The heart is conspicuously large with its distinct muscular fibers. It extends across the posterior end of the oesophagus (figs. 85, 88, *h.*).

The ovary is large and spherical and is located on the left side immediately posteriad of the stomach. The testis is elongated, with the anterior end very much wider than the posterior (fig. 88, *ov.*, *t.*). It lies on the right side of the ovary. In young animals both reproductive glands are rudimentary and spherical (fig. 87), but in older individuals they are greatly developed, the testis being composed of three or more large broken parts overlapping one another, and the ovary of a double sphere (fig. 88, *ov.*, *t.*).

It is interesting that at such a stage of over-developed reproductive glands much of the animal body appears to be gradually disappearing, probably by resorption. Of the entire complicated anterior portion of the trunk of such individuals the endostyle is the only structure that has any definite shape while the rest of the tissue is practically gone. A faint outline of the branchial openings is visible. The digestive system, while distinctly present, nevertheless shows signs of deterioration. If it were not for the characteristic tail and the posterior processes of the trunk, which are typical of the species, it would be difficult to identify such specimens as belonging to the species (fig. 88). This is not the only appendicularian that undergoes such changes with sexual development. It is very usual with *Althoffia* and *Stegosoma* that an over development of sexual glands is accompanied by gradual deterioration of the body.

The tail fins have nearly parallel edges to near the posterior end where they contract sharply to the broad bifurcation. The two pairs of large tail glands found in this species only, are situated on the ventral side symmetrically opposite on each side of the musculature. Many smaller glandular cells are scattered along both margins of the tail as well as throughout its musculature (fig. 86, *gl. c.*).

The species is large, 1.0 to 1.5 mm. in length not including the posterior processes. The width of the trunk is almost 0.33 of its length. The length of the tail is 1.25 times the length of the trunk. The width of the tail is 0.33 of its length. The width of the musculature is about 0.25 of the width of the tail and about twice the width of the chorda.

The species is found here only at rare intervals and then only in small numbers.

According to the records it has been found in the surface waters at the pier on the following dates:

Fritillaria pellucida

Date	Temperature	Number
February 21, 1920	13°4	2
February 22, 1920	13°4	2
February 24, 1920	13°9	3
March 4, 1920	13°0	1
March 8, 1920	12°8	2
March 10, 1920	13°4	1
January 17, 1921	13°83	1
January 31, 1921	13°76	2
February 18, 1921	14°29	1
April 20, 1921	14°24	1

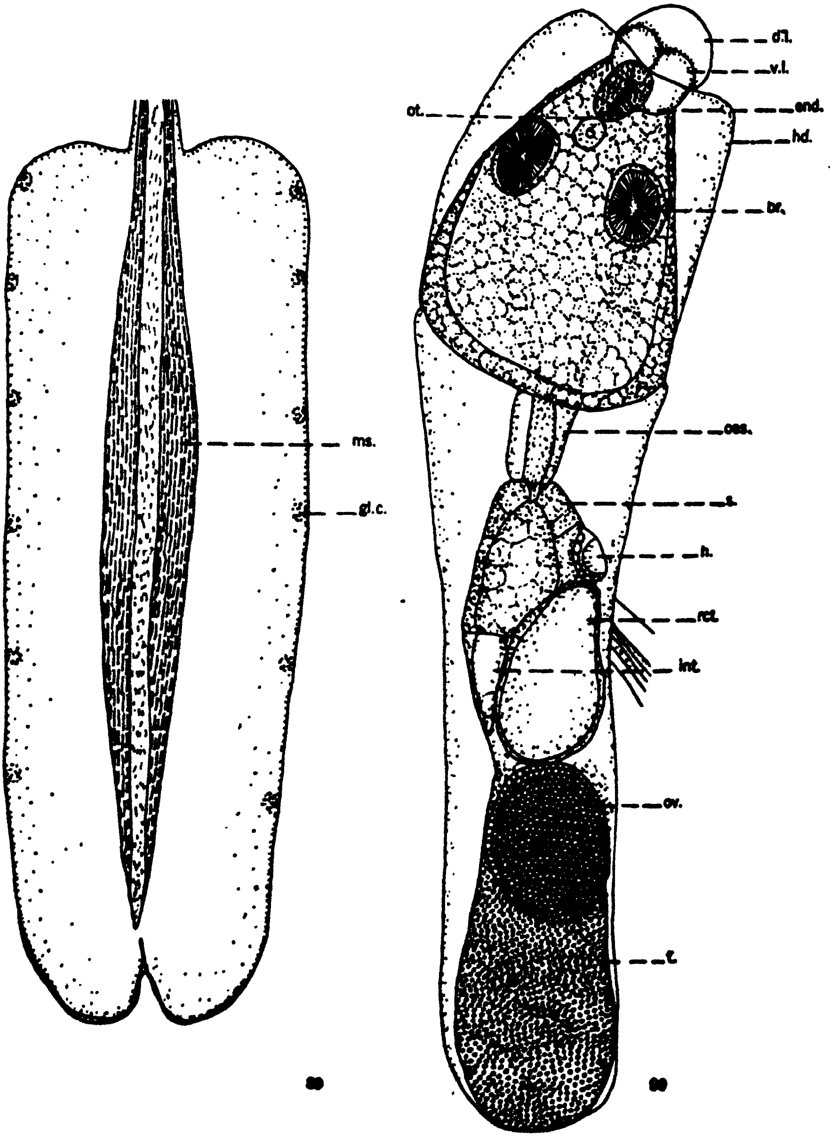
Two specimens were found in the surface waters near the Panama Canal on March 27 and 28, at temperatures 24° C. *Fritillaria pellucida* has been found near the Japanese coast (Aida, 1907). Fol (1872) frequently found it one year near the coast of Messina, December-April, while in the following year it was very rarely found there. Its distribution is known in all warm seas (Lohmann, 1896). It was found in the Florida stream (in August); in the Gulf stream (in October); near N. Ascension (in September); in the Atlantic Ocean near the entrance to the Mediterranean Sea; in the Zanzibar Canal; in various places in the Mediterranean Sea; and in the Indian Ocean, at surface temperatures of 18°9–28°5 C.

Although the exact depth from which *Fritillaria pellucida* was taken is not given, yet a statement has been made (Lohmann, 1896) that it was never found below 200 meters.

It seems doubtful that *Fritillaria pellucida* was actually found in the surface waters having a temperature as high as 28°5 C. If it has been found in the lower layers from 0–200 m., as is vaguely suggested, that temperature would naturally be considerably lower than the temperature recorded. During several years of observation in this region, no fritillarian has been found in the surface waters in a temperature above 17° C., but occasionally some are found in deeper layers even when the surface temperature is above 20° C.

Fritillaria gigas sp. nov.

Diagnostic characters.—A large species. Trunk slender with the anterior end broader than the posterior end; mouth simple, dorsal lip single, ventral lip divided into two lobes; endostyle straight and short;



Figs. 89–90. *Fritillaria gigas* sp. nov.

Fig. 89. Dorsal view of tail. × 80. Fig. 90. Dorsal view of trunk. × 160. Abbreviations: *br.*, branchial openings; *d.l.*, dorsal lip; *end.*, endostyle; *gl.c.*, glandular cells; *h.*, heart; *hd.*, hood; *int.*, intestine; *ms.*, musculature; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

branchial openings large, elongated, situated far apart; oesophagus short; stomach on the left side; rectum on the right; ovary spherical; testis long, twice the size of the ovary; tail parallel margined, narrowly bifurcated (fig. 89).

Fritillaria gigas is a large species. The anterior end of the body is considerably wider than the posterior end, and is surrounded by a large hood. A single dorsal lip and two shorter ventral lips, half the length of the dorsal surround the simple mouth (fig. 90, *d.l.*, *v.l.*). The endostyle is straight, short, broadly and regularly elliptical. On a level with its posterior end on the dorsal side is the otocyst (fig. 90, *ot.*). The large elongated branchial openings are located at a considerable distance posterior to the endostyle. The oesophagus between the pharynx and the stomach is short, thick-walled, and comparatively narrow (fig. 90, *oes.*). The stomach is on the left; elongated with the anterior end narrower than the posterior one. Its wall contains large cells. The rectum is on the right; elongated, almost parallel with the stomach, though it extends posteriad to the latter and slightly overlaps it on the ventral side. The annus points directly anteriad. The narrow intestine is dorsolaterally located and is simple without any accessory bodies. The heart is on the ventral side across the stomach (fig. 90, *h.*).

The reproductive glands occupy the posterior portion of the body. The large elliptical ovary lies directly posteriad to the rectum. It is followed by the large elongated testis which is twice the size of the ovary (fig. 90).

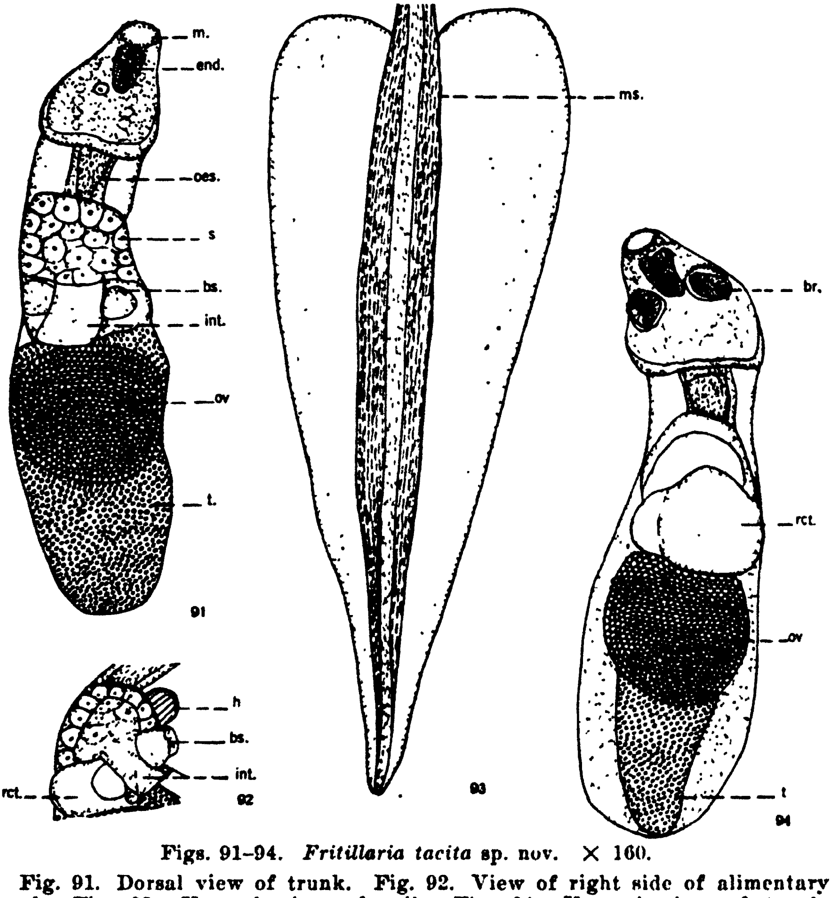
The length of the trunk is 0.96–1.2 mm. Its width is about 0.14 of the length (measuring across the stomach). The length of the tail is 1.5 times the length of the trunk with a uniform width of 0.33 its length. The tail is narrowly bifurcated, and the fins are rounded at both ends. A few glandular cells are scattered along the margin of the tail (fig. 89). The musculature is of medium width (fig. 89).

The species is not common in this vicinity. Only 7 specimens have been found in the surface hauls at the pier, on January 10, 12, and 15 at temperature 13.2–13.6 C.

Fritillaria tacita sp. nov.

Diagnostic characters.—A small species. Trunk stout; mouth simple, endostyle slightly curved; branchial openings circular, well separated from each other, and alongside the posterior end of the endostyle; oesophagus wide and short; stomach angular; ovary spherical, large, surrounded by the elongated testis; tail broad, tapering from near the base to a narrow point.

Fritillaria tacita is a small species. The trunk is slender, almost uniform in width, dorsoventrally compressed. The anterior end is triangular in shape (figs. 9, 94). The hood is very delicate. Only traces of it were left in the specimens examined. The mouth is simple, without projecting lobes. A row of refractile cells are found in the outer ridge of the mouth (fig. 91, *m.*). The posterior end of the endostyle is narrower than the anterior end and is turned slightly dorsad. The otocyst is small. The circular branchial openings, well separated from each other, lie alongside the endostyle at its posterior end near the lateral walls of the trunk. The oesophagus is wide and short (fig. 91, *oes.*). The stomach is wide and angular (fig. 91, *s.*). On the dorsal side, the intestine arises from the posterior end of the stomach. It possesses two blind sacs placed symmetrically one on each side (fig. 91, 92, *int.*, *bs.*). The rectum is on the ventral side. It is almost the size of the stomach. The heart extends obliquely across the ventral side of the stomach. The posterior half of the trunk is occupied by the reproductive glands, the large, spherical ovary lying immediately posterior to the rectum. The testis is about twice the size of the ovary, partly surrounding the latter, and extends posteriad, filling up the entire cavity (fig. 91, *t.*, *ov.*). In less developed stages, the testis is narrower and lies posterior to the ovary without surrounding it (fig. 94, *t.*, *ov.*).



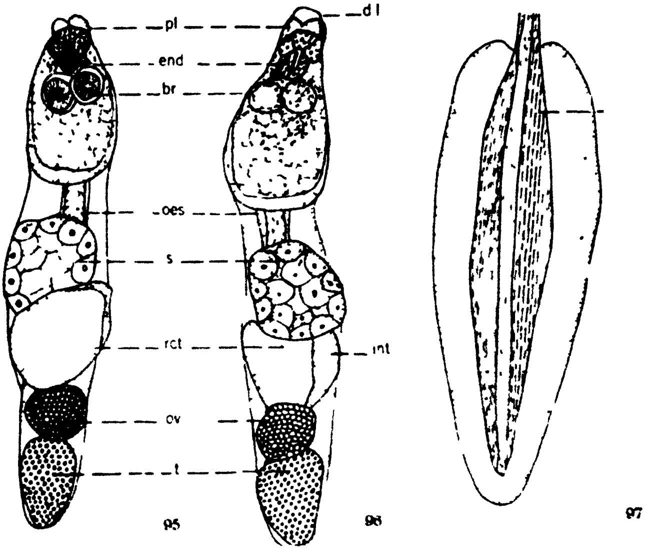
Figs. 91–94. *Fritillaria tacita* sp. nov. × 160.

Fig. 91. Dorsal view of trunk. Fig. 92. View of right side of alimentary canal. Fig. 93. Ventral view of tail. Fig. 94. Ventral view of trunk. Abbreviations: *br.*, branchial openings; *bs.*, blind sacs; *end.*, endostyle; *h.*, heart; *int.*, intestine; *m.*, mouth; *ms.*, musculature; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis.

The tail is broad at base and tapers gradually to a very narrow point. Its anterior fin ends are emarginate. The musculature is wide, about twice the width of the chorda (fig. 93).

The length of the trunk is about 0.5 mm. Its width is about 0.2 of the length. The length of the tail is about 1.4 times the length of the trunk. Its width is about 0.33 the length. The width of the musculature is about twice the width of the chorda.

The species is not frequently taken in this locality. Three specimens were found on January 3, 1922, temperature 14°75 C.



Figs. 95–97. *Fritillaria velocita* sp. nov.

Fig. 95. Ventral view of trunk. × 160. Fig. 96. Dorsal view of trunk. × 160. Fig. 97. Dorsal view of tail. × 80. Abbreviations: *br.*, *branchial* openings; *d.l.*, dorsal lip; *end.*, endostyle; *int.*, intestine; *ms.*, musculature; *oes.*, oesophagus; *ov.*, ovary, *pl.*, plasmatic structure; *rct.*, rectum; *s.*, stomach; *t.*, testis.

Fritillaria velocita sp. nov.

Diagnostic characters.—This is a small species. Trunk slender; mouth simple with a dorsal lip and two thick ventral lobes; endostyle nearly as broad as long; branchial openings circular, small, touching each other; oesophagus short; stomach angular; rectum large; ovary spherical; testis elongated; tail nearly elliptical, distal end rounded, anterior fin ends emarginate; musculature wide in the middle.

Fritillaria velocita is nearest related to ***F. borealis***, but differs from this in shape of the mouth, the relative position of the branchial openings, the shape of the tail, and in other minor details.

The trunk is very slender, almost uniform in width (figs. 95, 96) with a delicate hood. This was not in good condition in the specimen examined. The mouth is simple with a short dorsal lip (fig. 96, *d.l.*). From the extreme anterior end protrude two plasmatic structures similar to those in *F. borealis* (fig. 95, *pl.*). The endostyle is broad and short. The otocyst is small and inconspicuous. The circular branchial openings are close together, their walls being in apparent contact. They are immediately posterior to the endostyle (fig. 95, *br.*). The pharynx is comparatively large, and the oesophagus very short (figs. 95, 96, *oes.*). The stomach is angular and slightly elongated. The large pear-shaped rectum lies posterior to the stomach in an oblique position in relation to it. The two are connected by an intestine situated on the dorsal side (figs. 95, 96, *rct.*, *int.*).

The reproductive glands consist of a small spherical ovary situated immediately posteriad to the rectum, and an elongated testis about twice the size of the former (figs. 95, 96, *ov.*, *t.*). They occupy about 0.33 of the length of the trunk.

The tail is elliptical and terminates in a rounded tip, anterior fin ends emarginate. The musculature of the tail is wide, especially in the middle portion (fig. 97, *ms.*).

The length of the trunk is 0.43 mm. Its width is about 0.17 of the length. The length of the tail is 1.67 times the length of the trunk. The width of the musculature is nearly 3 times the width of the chorda.

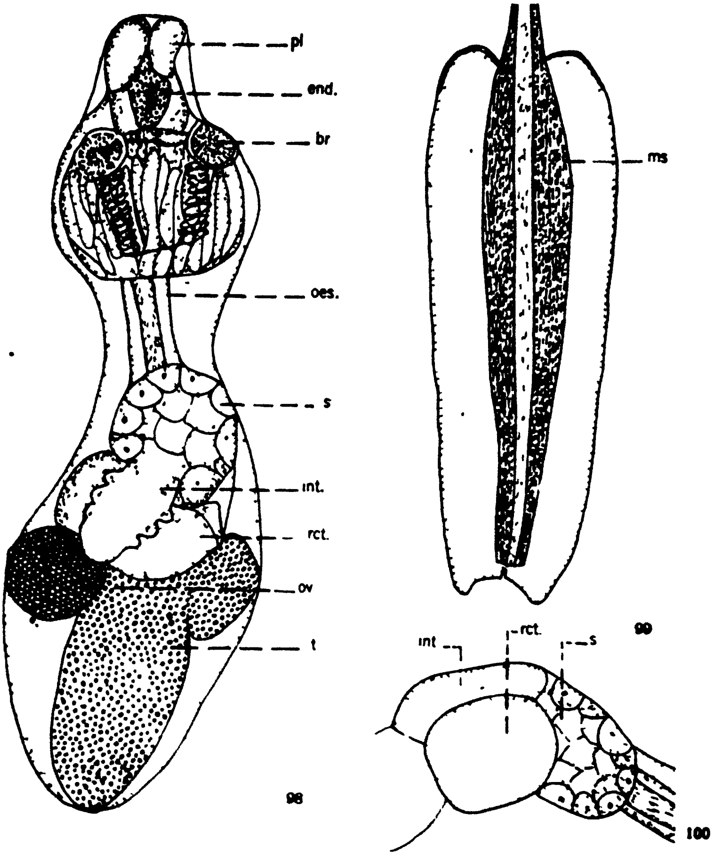
The species is occasionally found in the surface hauls of this vicinity. The last two specimens were found on January 4, 1922, temperature 14°43 C.

Fritillaria sargassi Lohmann (1896)

Diagnostic characters.—A large species. Trunk irregular in shape; large palp-like structures project anteriad from the mouth region; endostyle short; branchial openings circular, far removed from endostyle; otocyst inconspicuous; oesophagus thick-walled, medium length; stomach round; intestine wide and long with two pairs of large cells; rectum elongated; reproductive glands T-shaped; tail strap-shaped, emarginate at proximal end, bifurcate at the distal end; tail musculature wide.

Fritillaria sargassi is a large species that has been well described by Lohman (1896). It resembles *Fritillaria borealis* in many respects. The chief differences are found in the palp-like structures bordering the mouth (they are much longer and thicker in *Fritillaria sargassi* than in *Fritillaria borealis*); in the shape and arrangement of the reproductive glands; in the shape of the testis which is narrow and straight in *Fritillaria borealis*, while in *Fritillaria sargassi* it bulges out on the anterior right side pushing the ovary on the left thus forming a T and giving the posterior end of the trunk a broad appearance forming a strong contrast to the slender bodied *Fritillaria borealis;* in the outline of the musculature of the tail which ends in a rectangular fashion in *Fritillaria sargassi*, while in *Fritillaria borealis* it attenuates to a narrow point.

The mouth is surrounded by a dorsal and two ventral lobes. Between these, and attached to the inside of the ventral lobe, are two large opaque plasmatic masses (fig. 98, *pl.*) similar to structures found in other species of *Fritillaria* where they are richly supplied with flagella and cilia. However, in *Fritillaria sargassi* no cilia are found in connection with these structures. The endostyle is short, with its posterior end slightly turned up dorsad (fig. 98, *end.*). The branchial openings are circular and small, situated near the lateral walls of the trunk and far removed from the endostyle (fig. 98, *br.*); otocyst inconspicuous. The thick-walled oesophagus is of medium length and width. The stomach is round. From its dorsal end arises a wide conspicuous intestine, extending obliquely across the dorsal surface of the rectum (fig. 98, *rct.*). Large glandular cells, symmetrically placed, are on both sides of the intestine (fig. 98, *int.*). The elongated rectum is posterior to the stomach, slightly to one side and adjacent



Figs. 98–100. *Fritillaria sargassi* Lohmann.

Fig. 98. Dorsal view of trunk. × 160. Fig. 99. Dorsal view of tail. × 80. Fig. 100. Dorso-lateral view of alimentary canal. × 160. Abbreviations: *br.*, branchial openings; *end.*, endostyle; *int.*, intestine; *ms.*, musculature of tail; *oes.*, oesophagus; *ov.*, ovary; *pl.*, plasmatic structure; *rct.*, rectum; *s.*, stomach; *t.*, testis.

to it. Immediately posterior to the digestive organs are the reproductive glands. The great bulk of the testis extends posteriad to the end of the trunk. On the right side of its anterior end the testis sends out a branch. Opposite this branch, on the left side, is the spherical ovary forming the left cross bar of the T (fig. 98, *ov.*, *t.*).

The tail is nearly parallel-edged; it is bifurcate at the posterior end and emarginate at the anterior one. The musculature is broad, especially in the middle region, ending bluntly.

The length of the trunk is about 1 mm. The width of the trunk is about 0.33 of its length. The length of the tail is 1.4 times the length of the trunk. The width of the musculature in the widest part is nearly 4 times the width of the chorda.

The species occurs here during the winter months, between November and March, at irregular intervals. Some were taken in the surface hauls at the pier on the following dates:

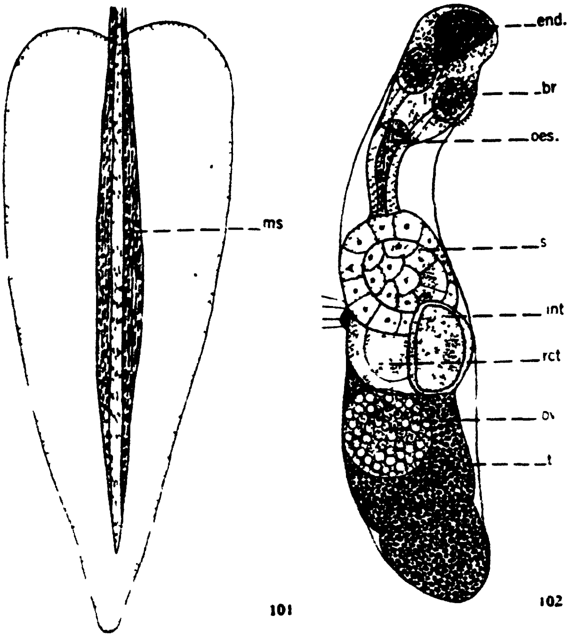
Fritillaria sargassi

Date	Temperature	Number
November 24, 1919	15°95	2
December 4, 1919	14°99	1
December 5, 1919	15°07	3
December 8, 1919	14°93	1
December 23, 1919	14°24	2
January 31, 1920	13°76	2
February 5, 1920	14°03	4
February 7, 1920	14°42	2
February 9, 1920	13°96	1
March 5, 1920	14°40	3
March 6, 1920	15°00	1
March 18, 1920	13°90	5
March 19, 1920	14°17	6
January 5, 1921	13°65	1
March 7, 1921	14°38	2
March 8, 1921	14°49	1
November 21, 1921	15°50	3
November 23, 1921	14°88	1
January 2, 1922	14°70	2

The distribution of *Fritillaria sargassi* extends throughout all warm areas (Lohmann, 1896). The species has been taken at all stations through the Equatorial stream region to Cape Verde, from August–September, but was absent from the cold island region of North Ascension. Lohmann (1896) further reports its occurrence from the Canary Island region, from the Indian Sea, from the Canal of Zanzibar and from the North Sea. *Fritillaria sargassi* was found at the various localities at a surface temperature varying from 19°8–28°5 and in depths from 0–900 meters. Some were taken (evidently in closed nets) from depths of 700–900 m. at a temperature of 4° C.

Fritillaria truncata sp. nov.

Diagnostic characters.—This is a small species. Trunk slender and uniform in width; mouth simple; endostyle broad, short; branchial openings small, circular; oesophagus narrow and short; stomach large and angular; ovary spherical; testis elongated; tail broad anteriorly, wedge-shaped, tapering to a narrow point.



Figs. 101-102. *Fritillaria truncata* sp. nov.

Fig. 101. Dorsal view of tail. × 80. Fig. 102. Dorsolateral view of trunk. × 160. Abbreviations: *br*, branchial openings; *end.*, endostyle; *int.*, intestine; *ms.*, musculature of the tail, *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s*, stomach; *t.*, testis.

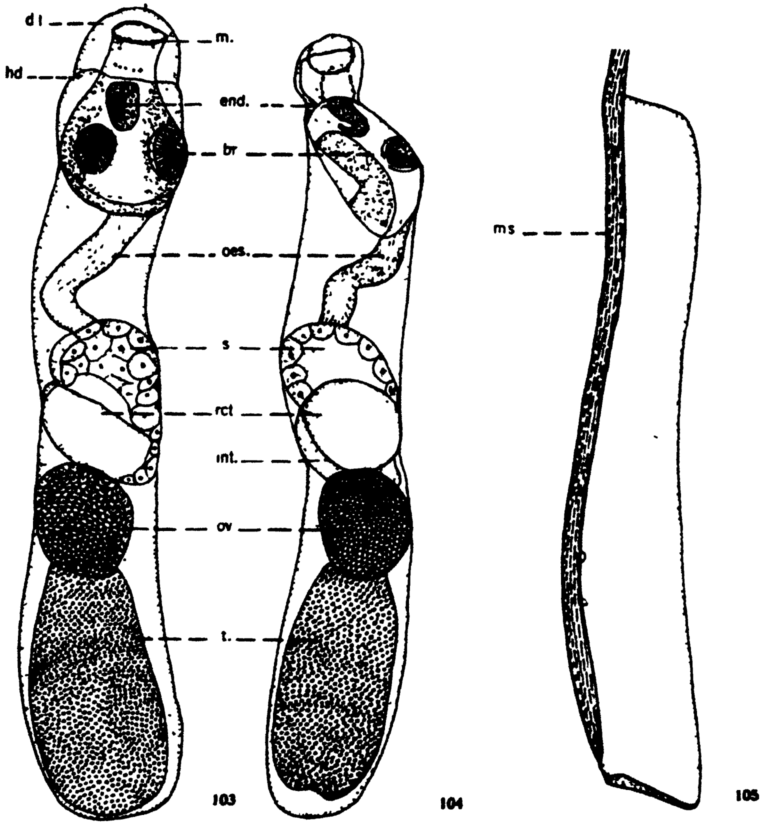
The anterior end of the trunk is round, narrow, club-shaped. No hood was visible. The endostyle is very broad and short, rapidly narrowing toward the posterior end (fig. 102, *end.*). The small, circular branchial openings are near together but not in contact and are posteriad of the endostyle (fig. 102, *br.*). The oesophagus is narrow and short. The somewhat quadrilateral, large stomach is situated nearly in the main axis of the body. The rectum is elongated and lies obliquely posteriad of the stomach, partly overlapping it (fig. 102, *s.*, *rct.*). The intestine is smooth, without accessory bodies, and is located on the dorsal side (fig. 102, *int.*).

The long, narrow posterior end of the trunk is occupied by the reproductive glands, a spherical ovary and elongated testis. The latter is about twice the size of the ovary which it partly surrounds anteriorly (fig. 102, *ov.*, *t.*). The cigar-shaped testis in mature state may form in two parts lying adjacent to each other.

The fins of the tail are wide at the proximal end and gradually taper to a narrow point at the distal end. They are slightly emarginate anteriorly. The musculature of the tail is narrow (fig. 101).

The length of the trunk is 0.5 mm. The width is less than 0.2 of its length. The tail is about 1.67 the length of the trunk. Its width is about 0.33 of the length. The musculature is about twice the width of the chorda.

Only 2 specimens were found in the surface hauls at the pier on December 30, 1921, temperature 15°2 C.



Figs. 103–105. *Fritillaria dispara* sp. nov.

Fig. 103. Ventral view of trunk. × 80. Fig. 104. View of right side of trunk. × 80. Fig. 105. Side view of the tail. × 40. Abbreviations: *br.*, branchial openings; *end.*, endostyle; *hd.*, hood; *int.*, intestine; *m.*, mouth; *ms.*, musculature; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s*, stomach; *t.*, testis.

Fritillaria dispara sp. nov.

Diagnostic characters.—A large species. Trunk very slender; mouth simple; endostyle broad at anterior end, posterior end turned up; branchial openings small and circular; pharynx short; oesophagus long; stomach round; ovary spherical; testis elongated; tail long and parallel edged to the very end.

Fritillaria dispara is a large species, which possesses a trunk that is unusually narrow and long. The hood is small and delicate (fig. 103, *hd.*). There is a large round dorsal lip (fig. 103, *d.l.*). The mouth is simple, surrounded by a ridge of refractile cells (fig. 103, *m.*). The endostyle is short with its anterior end broad and its posterior end narrow and turned in a sharp angle dorsad (figs. 103, 104, *end.*). The circular branchial openings are located near the lateral walls widely separated from each other and from the endostyle (fig. 103, *br.*). The oesophagus is narrow and exceptionally long and the pharynx is comparatively short (figs. 103, 104, *oes.*). The stomach is oval in shape. A smooth intestine connects the stomach and rectum (figs. 103, 104, *s.*, *int.*, *rct.*).

The long space posteriad of the rectum is occupied by the reproductive glands. They consist of a spherical ovary and a long testis about three times the size of the former, and thickest at the posterior end.

The tail is quite unique in the parallelism of its edges and the broad straight edged notch in which it ends posteriorly. Its musculature is relatively narrow and uniform in width (fig. 105).

The species is larger than the average *Fritillaria*. The length of the trunk is 1.45 mm. The width is about 0.12 of its length. The length of the tail is nearly twice the length of the trunk. Its width is less than 0.33 its length. The width of the musculature is less than twice the width of the chorda.

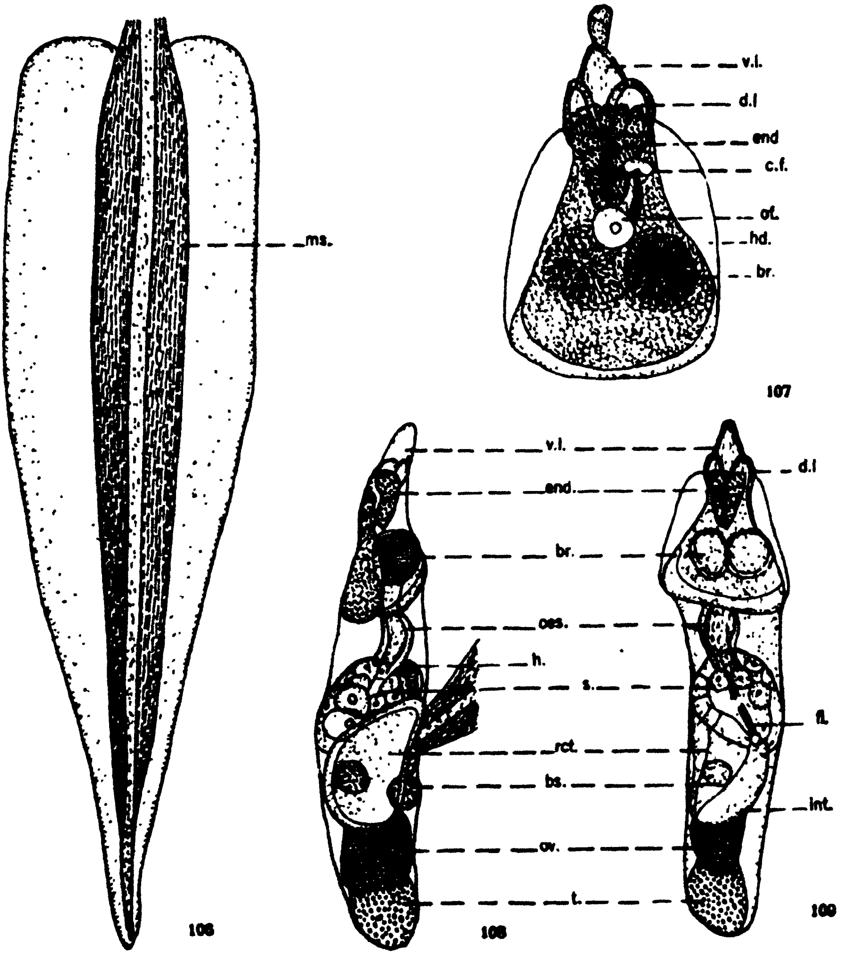
Two specimens of *Fritillaria dispara* were found in the surface waters near San Pedro in the winter of 1920.

Fritillaria tereta sp. nov.

Diagnostic characters.—A small species. Trunk dorsoventrally compressed and slender; mouth surrounded by a transparent membranous dorsal lip and two ventral ones provided with heavy ridges; endostyle unusually large; otocyst and ciliated fossa conspicuous; branchial openings very large and circular; oesophagus short; stomach angular; intestine long with two blind sacs; rectum elongated; ovary and testis single, round; tail long, cordate, acuminate, anterior end emarginate; musculature broad.

Fritillaria tereta is a small and distinctive species, and differs so entirely in several essential characteristics from any of the other *Fritillaria* that it is easily identified. It is nearest related to *Fritillaria lohmanni* in that both species have a single ventral lip and a bilobed dorsal one, while in most species of *Fritillaria* the reverse is true. (See figs. 11, 20, 107, 108.) The trunk is dorsoventrally compressed and narrow. The posterior end especially appears narrow in comparison to the broad anterior one in ventral view. The anterior portion of the trunk is triangular with the broad plasmatic ridge forming the base of the pharynx and the anterior portion of the triangle. From this base both sides of the walls run slantingly toward the mouth. The triangle comes to an actual sharp point at the tip of the ventral lip.

The ventral lip is broad at the base becoming narrower toward the anterior end. The dorsal lip is membranous and transparent, divided by a deep median notch into two rounded lobes. These, as well as the ventral lip, are bordered by heavy ridges with scattered refractile cells (fig. 107, *v.l.*, *d.l.*). From the tip of the ventral lip projects a membrane with cilia. The mouth is also provided with a heavy ridge from which large, papilla-like cells project. The endostyle is unusually large and very wide at its anterior end, while the posterior end is much narrower. The outline of the endostyle is regularly triangular (figs. 107, 109, *end.*).



Figs. 106–109. *Fritillaria tereta* sp. nov.

Fig. 106. Dorsal view of tail. × 160. Fig. 107. Dorsal view of anterior portion of trunk. × 320. Fig. 108. View of right side of trunk. × 160. Fig. 109. Dorsal view of trunk. × 160. Abbreviations: *br.*, branchial openings; *bs.*, blind sacs; *c.f.*, ciliated fossa; *d.l.*, dorsal lip; *end.*, endostyle; *fl.*, flagella; *h.*, heart; *hd.*, hood; *int.*, intestine; *ms.*, musculature; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

The circular branchial openings are very large, occupying the entire width of the trunk, with their median walls in contact. The otocyst and the ciliated fossa are conspicuous on the dorsal side (fig. 107, *ot.*, *cf.*). The oesophagus is short (figs. 108, 109). The heart is very conspicuous (fig. 108, *h.*).

The stomach is large, narrower at its anterior end. The opening of the oesophagus into the stomach is armed with a tuft of long cilia, in the usual fashion for the genus; but in addition at the opposite end of the stomach where the latter meets with the intestine, there is another tuft of cilia (fig. 109, *fl.*) which is unusual for the genus. The intestine is narrow and provided with two accessory bodies or blind sacs (fig. 108, *bs.*). The rectum is large, elongated, slightly curved. The ovary is elongated, rectangular in shape. The testis is of equal size with the ovary and is immediately posterior to it (figs. 108, 109).

The tail is long, cordate, wide anteriorly and tapers very gradually to a narrow end, anterior end emarginate. The musculature is wide, narrowing toward the end (fig. 106).

The length of the trunk is about 0.4 mm. Its width is about 0.25 its length. The length of the tail is about 1.67 times the length of the trunk. Its width is 0.25 of its length. The width of the musculature in its widest part is about 4 times the width of the chorda.

The identification is based on a single specimen. Some of the illustrations were outlined while the animal was living and it was possible to watch all the organs at work. A hood was present. On account of its very small size and transparent condition this appendicularian may be easily overlooked. The one individual here described was found on January 3, 1922, in the surface hauls at the pier, at a temperature of 14°7 C.

Fritillaria delicata sp. nov.

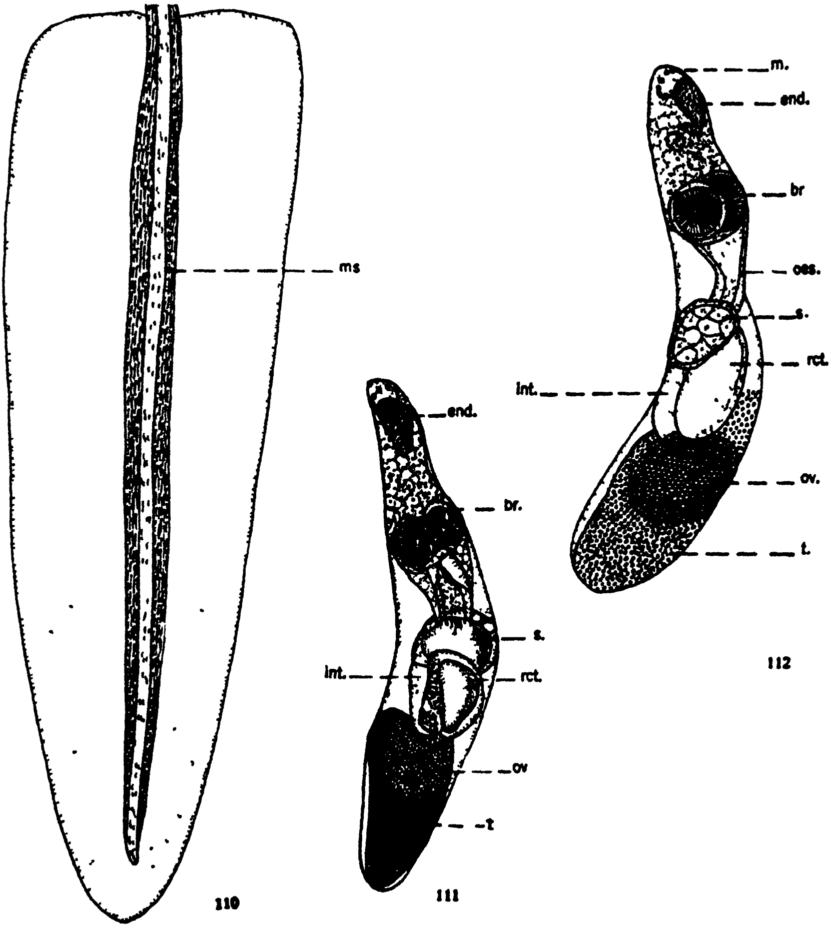
Diagnostic characters.—A small species. Trunk slender and long; mouth simple; endostyle long; branchial openings large, far posterior to the endostyle; oesophagus short; stomach round; intestine smooth, without accessory bodies; rectum large, elongated; ovary elliptical; testis long, almost three times the size of the ovary; tail long, triangular, rounded at posterior end, the fins truncate at anterior end.

Fritillaria delicata is a small species, the trunk of which is very narrow and almost uniform in width (figs. 111, 112). No lips are present but the simple mouth is surrounded by a ring of large refractile cells (fig. 112, *m.*). The endostyle is long and narrow, the anterior half somewhat broadest, the posterior end narrow and bending dorsad (fig. 112, *end.*). The branchial openings are large and circular, touching each other in the median line. They are far behind the endostyle and occupy the entire width of the pharynx. The oesophagus is short and broad (fig. 112, *br.*, *oes.*).

The stomach is slightly elongated transversely to the axis of the body. It is followed posteriad by the large elongated rectum. The narrow intestine is on the dorsal side. It is smooth, without any accessory bodies (figs. 111, 112, *s.*, *rct.*, *int.*).

The reproductive glands occupy 0.33 of the posterior portion of the trunk. They consist of an elongated, large ovary and a long testis almost three times the size of the ovary posteriad of this and partly surrounding it (figs. 111, 112, *ov.*, *t.*).

The tail is long, triangular with rounded posterior end and truncated anterior end. Its musculature is narrow (fig. 110, *ms.*).



Figs. 110–112. *Fritillaria delicata* sp. nov. × 160.

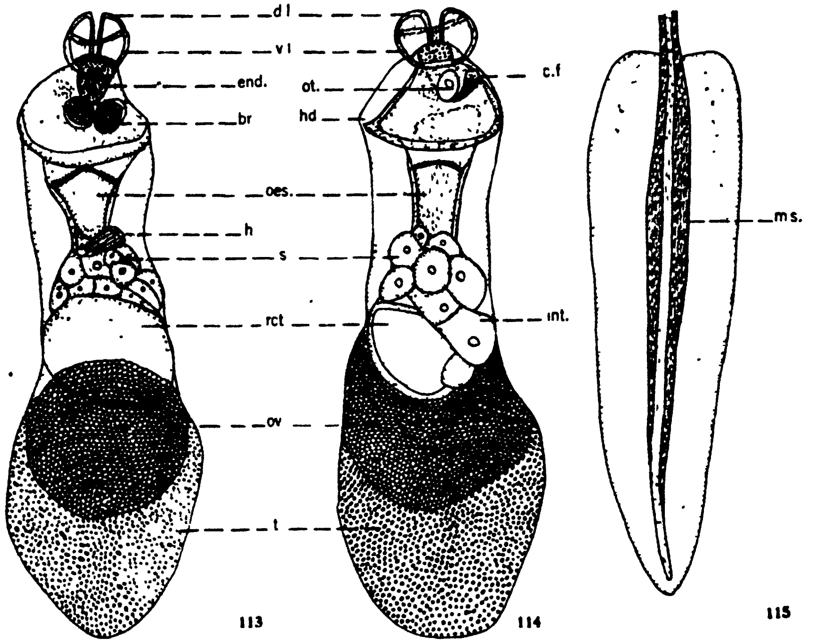
Fig. 110. Dorsal view of tail. Fig. 111. Ventral view of trunk. Fig. 112. Right side view of the trunk. Abbreviations: *br.*, branchial openings; *end.*, endostyle; *int.*, intestine; *ms.*, musculature of tail; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis.

The length of the trunk is 0.43 mm. Its width is about 0.14 of its length. The length of the tail is about 1.7 times the length of the trunk. The width is about 0.33 of its length. The musculature of the tail is uniformly narrow measuring about 1.5 times the width of the chorda.

The species is rare in this region. Five specimens were taken in the surface hauls at the pier on December 28 and 29, 1921, at a temperature of 14°62 and 14°80 C. It has not been seen since that date.

Fritillaria brevicollis sp. nov.

Diagnostic characters.—A small species. Trunk broad, sac-shaped, its posterior end expanded by bulky reproductive glands; ventral lip broad, single; dorsal lip bilobed; mouth simple; branchial openings small, circular, near together and close posterior to the endostyle; oesophagus broad; stomach irregular in outline due to the large cells; ovary and testis single; tail tapering gradually to the blunt distal end.



Figs. 113–115. *Fritillaria brevicollis.*

Fig. 113. Ventral view of trunk. × 160. Fig. 114. Dorsal view of trunk. × 160. Fig. 115. Dorsal view of tail. × 80. Abbreviations: *br.*, branchial openings; *c.f.*, ciliated fossa; *d.l.*, dorsal lip; *end.*, endostyle; *hd.*, hood; *int.*, intestine; *ms.*, musculature of tail; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

Fritillaria brevicollis is easily identified by the shape of the trunk, which is characteristic of the species. It is dorsoventrally compressed, almost uniform in width, except the posterior end which is expanded by the large reproductive masses. The antérior, branchial region of the trunk is very short, almost forming a triangle (figs. 113, 114). The hood is well developed. The mouth is simple. The thin, membranous lips with thick ridges resemble, in general the corresponding structures in *Fritillaria lohmanni* and *Fritillaria tereta*. Similar to those species the ventral lip is single but differs from that of the others in that it has a concave anterior edge. In this heavy ridge are imbedded a number of conspicuous refractile cells (fig. 113, *v.l.*). The dorsal lip consists of two lobes with the central edge running parallel and each forming a sharp angle from which the outer ridges run posteriad, each forming a crescent on the outer side of the lobe (fig. 114, *d.l.*). The endostyle is broad and short (fig. 113, *end.*). The otocyst and ciliated fossa are conspicuous (fig. 114, *ot.*, *c.f.*). The circular branchial openings are unusually small. They are crowded together in the center immediately posterior to the endostyle (fig. 113, *br.*). The oesophagus on the whole is wide with its posterior end narrower than the anterior one (figs. 113, 114, *oes.*). The heart is on the ventral side partly covering the oesophagus and the anterior end of the stomach (fig. 113, *h.*).

The stomach is round. Its walls are composed of large cells, giving it an irregular outline (figs. 113, 114, *s.*). The narrow intestine extends obliquely across the large rectum on the dorsal side and has a pair of blind sacs (fig. 114, *int. rct.*). The reproductive glands occupy the posterior end of the trunk filling the large cavity. The ovary is large and spherical, overlapping the large rectum. The broad, short testis lies posteriad of the ovary and partly surrounds it (figs. 113, 114, *ov.*, *t.*).

The tail is long-triangular but somewhat rounded at the broad tip. Its musculature is narrow, occupying about 0.2 of the entire width of the tail, and terminating short of the tip of the chorda (fig. 115, *ms.*).

The species is of small size. The length of the trunk is about 0.5 mm. The width is 0.2 of the length. The length of the tail is 1.67 times that of the trunk. The width of the tail is about 0.25 of its length. The width of the musculature is about twice the width of the chorda.

Fritillaria brevicollis, like most *Fritillaria*, is rare on this coast. Nine specimens were found in the surface waters at the pier on November 28, November 30, and December 2, 1921, temperatures 15°63, 15°02, and 14°98 C. respectively.

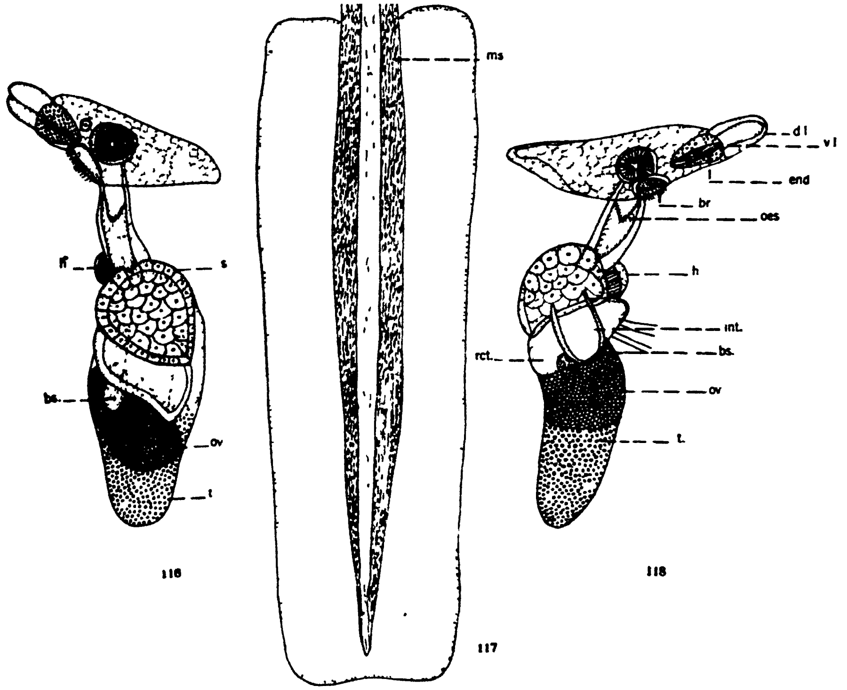
Fritillaria claudaria sp. nov.

Diagnostic characters.—This is a small species. Trunk laterally compressed; its anterior portion terminating in a peculiar blind sac; mouth simple, provided with a dorsal and a ventral lip; endostyle broad, especially anteriorly; branchial openings circular, large, a short distance posterior to the endostyle; oesophagus broad, long; stomach heart-shaped; intestine with two blind sacs; rectum pear-shaped; ovary single, elongated; testis long, partly surrounding the ovary; tail nearly parallel edged, truncate at posterior end, its musculature narrow.

Fritillaria claudaria, though a small species, is easily identified by its peculiarly shaped trunk, which is so much compressed laterally that it is almost impossible to place the animal on its back, or to obtain a good ventral or dorsal view. The anterior, branchial region of the trunk forms posteriorly a blind sac. This part of the body is often seen in a position at right angles to the rest of the trunk (figs. 116, 118). In this respect the species differs from all other known *Fritillaria*. The simple mouth is provided with dorsal and ventral lips, both of which are transparent, narrow and undivided (fig. 118, *d.l.*, *v.l.*). The endostyle is short and wide, with its posterior end narrower.

It is almond-shaped in outline (fig. 118, *end.*). The otocyst is small but distinct. The branchial openings are large and circular, located posterior to the endostyle, without touching it (fig. 118, *br.*). The oesophagus is comparatively wide and long, strongly ciliated (fig. 118, *oes.*). The heart is conspicuous (figs. 116, 118, *h.*).

The stomach is large and regular, heart-shaped (fig. 116, *s.*). The rectum is pear-shaped (fig. 118, *rct.*). The intestine is distinctly smaller than the rectum and its length is at right angles to the length of the rectum. It has two blind sacs placed symmetrically opposite (fig. 118, *bs.*).



Figs. 116–118. *Fritillaria claudaria* sp. nov. × 160.

Fig. 116. View of left side of trunk. Fig. 117. Dorsal view of tail. Fig. 118. View of right side of trunk. Abbreviations: *br.*, branchial openings; *bs.*, blind sacs; *d.l.*, dorsal lip; *end.*, endostyle; *h.*, heart; *int.*, intestine; *ms.*, musculature; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *v.l.*, ventral lip.

The compact ovary is followed immediately and partly surrounded by the larger testis (figs. 116, 118, *ov.*, *t.*). The tail is almost uniform in width and truncate with a slight identation at its tip. Its anterior fin ends are nearly truncate but slightly emarginate.

Fritillaria claudaria is a small species. The length of the trunk is 0.46 mm. The width is about 0.17 of the length. The length of the tail is 1.5 times that of the body. Its width is 0.25 the length of the tail. The musculature is about 0.2 of the entire width of the tail, and twice the width of the chorda.

Only two specimens were found after a stormy day, in the surface waters at the pier on December 30, 1920, temperature 13°38 C.

Fritillaria formica Fol (1872)

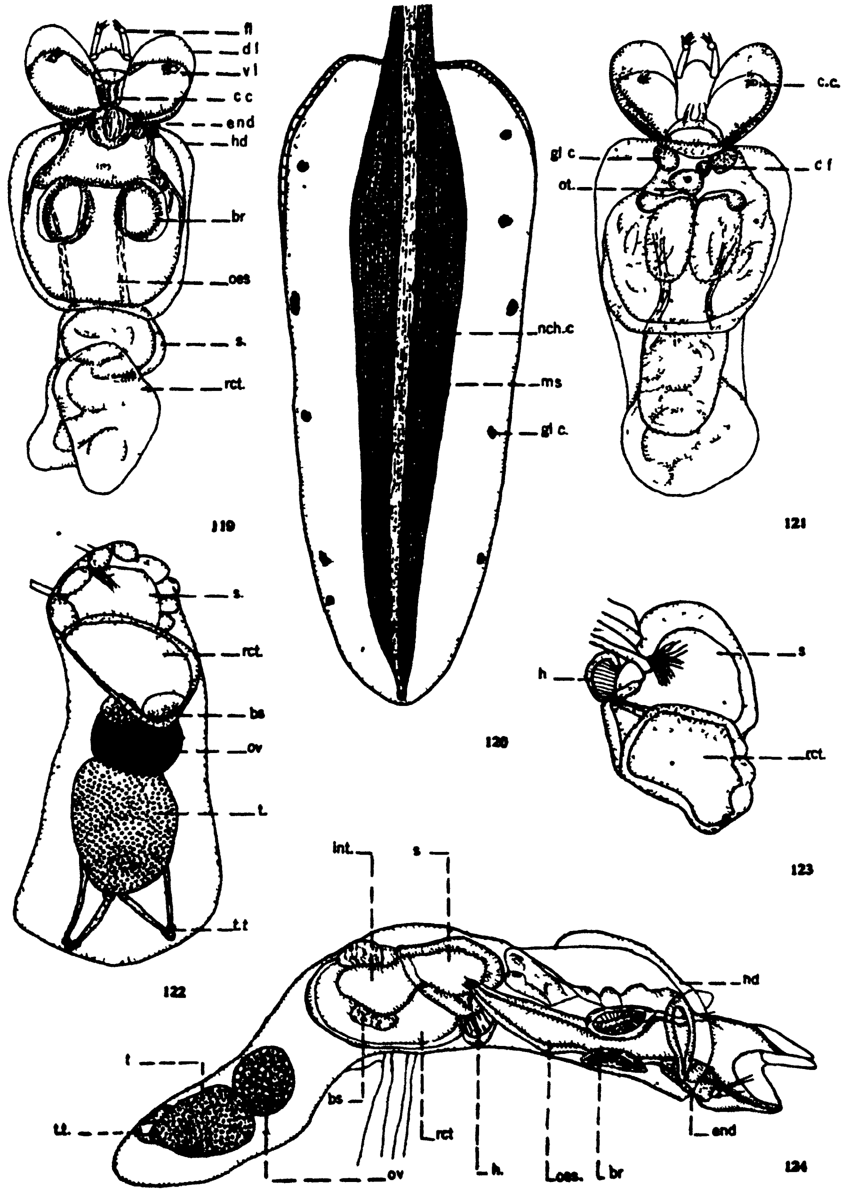
Diagnostic characters.—A medium-sized species. Trunk slender with both ends turned ventrad; mouth surrounded by a complicated system of cilia and by six lobes; endostyle short, with both ends turned dorsad; branchial openings elongated; oesophagus long; otocyst, ciliated fossa, and heart conspicuous; stomach, heart-shaped; intestine provided with large blind sacs; ovary and testis single; from the testis posteriad protrude one to two pairs of cord-like structures or tubes; tail with wide musculature and many gland cells.

Fritillaria formica is easily distinguished by its bent trunk, both extremities of which are turned ventrad, forming a hump in the middle on the dorsal surface (fig. 124). The mouth is surrounded by two pairs of large lobes (fig. 119, *d.l.*, *v.l.*). These lobes spread out laterally having a wide concavity from which arise two smaller lobes, one on the ventral, the other on the dorsal side, making six lobes in all. From the anterior end of the median dorsal lobe arise two finger-like projections, each bearing a tuft of long cilia at their tips (fig. 119, *fl.*). The median ventral lobe is less conspicuous than the dorsal one. Near the ridge of the ventral lobes, on the inside, are two large cilia-bearing cells, one on each side (fig. 121, *c.c.*). There is a row of cilia on the ventral side of the ridge of the mouth. In the center of the ventral side above the anterior margin of the endostyle is a group of large cells from which project long cilia (fig. 119, *c.c.*). All these cilia and flagella beat rhythmically in the living animal. The endostyle is short and wide with both ends turned dorsad (fig. 119, *end.*). Beneath the endostyle is a pair of large glandular cells one on each side (fig. 121, *gl. c.*). The otocyst, ciliated fossa, and heart are prominent (fig. 121, *ot.*). The ciliated fossa is small (fig. 121, *c.f.*). The branchial openings are large, circular or slightly elongated, and are far posteriad to the endostyle. The oesophageal opening into the stomach is provided with a long tuft of cilia (figs. 122, 123); oesophagus long.

The alimentary canal consists of a somewhat heart-shaped stomach situated antero-dorsad of the rectum. Near its ventral upper corner is the conspicuous heart (figs. 123, 124, *h.*). On the right side of the postero-ventral region of the stomach the intestine arises leading into the large rectum. On each side of the intestine is a large blind sac (fig. 124, *bs.*).

The posterior portion of the body is sac-shaped, widening toward the posterior end. The ovary is spherical. The testis may be spherical or elongated. The latter is usually the shape with older, more mature animals. From the posterior end of the testis project posteriad one or two pairs of long, tube-like structures, ending in an enlargement or in a group of cells (figs. 122, 124, *t.t.*). Whether or not these are sperm ducts has not been ascertained.

The tail is wide, with cells scattered near the margin on both wings of it (fig. 120 *gl. c.*). It narrows slightly distally and terminates in a rounded tip. The fins are also rounded at their anterior ends. The musculature is wide, occupying about one-third of the entire width of the tail (fig. 120, *ms.*). The notochordal cells are conspicuous (fig. 120, *nch. c.*).



Figs. 119–124. *Fritillaria formica.*

Fig. 119. Ventral view of anterior portion of trunk. × 160. Fig. 120. Dorsal view of tail. × 80. Fig. 121. Dorsal view of the anterior portion of trunk. × 160. Fig. 122. Ventral view of the alimentary canal and of the reproductive glands. × 160. Fig. 123. View of left side of the alimentary canal. × 160. Fig. 124. View of right side of trunk. × 160 Abbreviations: *br.*, branchial openings; *bs.*, blind sacs; *c.c.*, ciliated cells; *c.f.*, ciliated fossa; *d.l.*, dorsal lip; *end.*, endostyle; *fl.*, flagella; *gl.c.*, glandular cells; *h.*, heart; *hd.*, hood; *int.*, intestine; *ms.*, musculature; *nch. c.*, notochordal cells; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *s.*, stomach; *t.*, testis; *t.t.*, tubes of the testis; *v.l.*, ventral lip.

The size of the trunk varies from less than 0.4 mm. to over 1 mm. in length. The width of the trunk is about 0.25 its length and the length of the tail is about 1.5 times the length of the trunk.

Fritillaria formica is one of the most common species in this region, and has at times occurred in great numbers. During February 1–5 and February 21 to March 8, 1918, it was found daily in the surface catches at the pier, 50–200 individuals per catch, at temperatures varying from 12°3–14°5. After that date the numbers were low until March 15, when suddenly the number rose to 110 per catch. On that day the surface temperature dropped to 11°7, which indicates that up-wellings of colder waters or some similar change had taken place in the ocean water. On the following day only 5 individuals per catch were found and the temperature had risen to 13°9 C. After March 20, all *Fritillaria formica* had disappeared and were not seen again until November 4, the following year. *Fritillaria formica* appeared again in great numbers, 100–150 per catch on March 18 and 19, 1920, at temperatures of 13°85 and 14°17. After that date the number dropped to zero.

It would occupy too much space to give the detailed data for each day. *Fritillaria formica* is found almost every day in the surface waters near the pier during the winter months November–March. However, even during this season it may be absent for several days or weeks in succession, after which it may suddenly appear in great quantities, 150 ± per catch. Ordinarily the number varies from 1–20 individuals.

Fol (1872) found the species frequently in the Mediterranean Sea, near Messina, from December, 1869 to March, 1870, but did not find it there in 1871. Lohmann (1896) reports the occurrence of *Fritillaria formica* in all warm water areas where it is most regularly found. Near the shores of Bermuda it was taken from August–October, no other species of *Fritillaria* being found there during that period. The most northern areas mentioned in which *Fritillaria formica* was found were the Florida stream on August 3, and in the Gulf stream, north of the Azores, in October. It was found in abundance near the shores of West Africa in November, at surface temperature of 28°5; in the Gulf of Guinea, in November, surface temperature 28°9; near Madeira, from May to September; and in the Atlantic Ocean at the entrance to the Straits of Gibraltar. According to this report (Lohmann, 1896) the species was found in surface temperatures varying from 17°6–29°7 C. It was never found below a depth of 200 meters. It is not stated whether these appendicularians were taken from the surface waters in high temperatures, or from deeper layers. In the San Diego region *Fritillaria formica* was found in surface waters at temperatures 11°7 to 17°3 C. A few individuals were taken from depths of 150 meters in July, 1921, when the surface temperature was 22°–23° C. It is surprising that this species of such wide distribution was not found by Aida in the Japanese waters.

Subfamily *Oikopleurinae* Lohmann (1896)

Trunk stout. Mouth semicircular without projecting lobes but with prominent semicircular underlip. Endostyle never turned up but straight, made up of many cells. Branchial openings far removed from the mouth, in the region of the rectum. Oesophagus ascending to the dorsal side of the stomach and entering there; the walls of the stomach composed of three different kinds of epithelial cells: the small ciliated cells, the small glandular cells, and the large glandular cells, the latter arranged in rows. In most genera, stomach with two lobes (a left and a right) joined together to a greater or less extent. Intestine varying in length in different genera and species, and without blind sacs. Rectum following intestine. Shape and size of gonads varying in different genera and species. Oikoplast epithelium covering greatest part of the trunk extending posteriad in some species so far as to cover part of the gonads. Distal end of tail never bifurcated.

This family has from four to five genera but the species are less numerous than are those of Fritillarinae.

Genus **Oikopleura** Mertens (1831)

All the species of the genus *Oikopleura* are very much alike in their external appearance. The classification of the species is based chiefly on the shape and relative position of the digestive organs.

Diagnostic characters.—Trunk ovoid; no hood present; mouth with semicircular ventral lip; endostyle straight; external branchial openings on the ventral side of the body, internal end of branchial tubes opens into the pharynx at its base, these tubes being on each side of the rectum; oesophagus entering left stomach at upper end; stomach with two large lobes (right and left), connected by a middle piece along entire length; left stomach usually provided with a number of large gland cells arranged in an order characteristic of each species; intestine arising from the posteroventral end of the right stomach leading into a rectum; gonads posterior to digestive system or in some species extending anteriad and covering the alimentary canal on both sides; tail long, three to five times the length of the body.

Mouth is always simple with a semicircular ventral lip. The endostyle is short, straight, never bent. It always has two strong, flattened cirri at its anterior end, beating rhythmically from time to time. The branchial openings are round with their exterior openings near the base of the tail. The digestive system is very much crowded together. The oesophagus is much turned. The stomach and rectum are in immediate contact with the posterior ventral portion of the pharynx, while the curved intestine arises from the posterior ventral end of the right stomach, which leads into the rectum. The shape and relative position of the digestive organs is constant in the same species but is hardly ever alike in two different species. The gonads may lie posterior to the digestive system, or, in some species extend anteriad, covering the alimentary canal on both sides. The tail is long, three to five times the length of the body. The heart is situated on the ventral side over the right lobe of the stomach. Its long muscular fibers are placed longitudinally or posteroanteriorly, and beat with great rapidity. The nervous system is in general the same as in the group of *Copelata* described on previous pages. The ovaries and testis usually occupy the posterior region of the trunk, but they may extend to both sides.

The transparent muscilaginous house may reach an enormous size but in this region the house of the *Oikopleura* is usually small. It differs from the house of the Fritillaria in that it extends dorsad while in *Fritillaria* the house is formed beneath the hood at the extreme anterior end of the trunk and extends anteriad.

Every few hours a new house is secreted from the large, thick ectothelium cells which are restricted to the anterior end of the trunk extending as far posterior as to the insertion of the tail, sometimes covering the digestive system. These oikoplast epithelium cells are arranged in a pattern characteristic to each species. Near the anterior end on the dorsal side extend laterally two transverse rows of large elliptical cells forming two oval or elliptical figures one on each side of the dorsal line. These are surrounded by many rows of smaller cells arranged in a pattern characteristic to each species. These cells are usually referred to as the oikoplast zone and are known to secrete the house and the threads or meshwork which guard the entrance to the house through the two openings lying in this region. In many species of *Oikopleura* a group of large cells is also found on both sides near the stomach and is known as "Eisen's oikoplast." Posterior to the insertion of the tail the trunk is covered by a thin smooth epiderm which has very little if any secreting power.

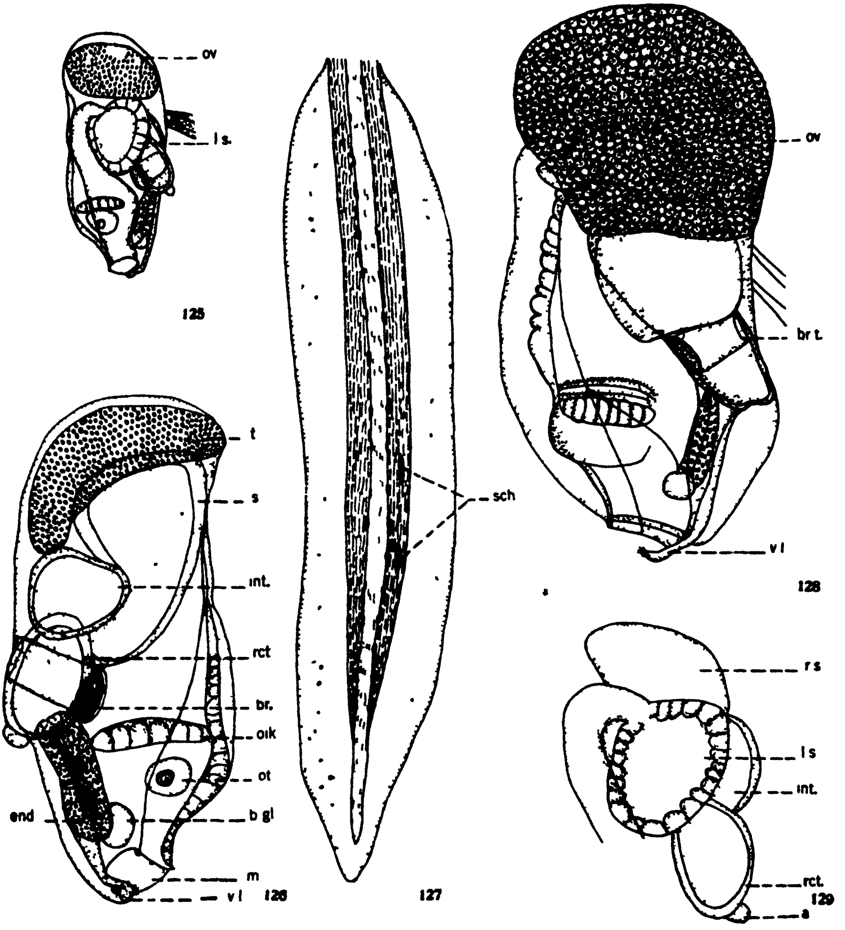
The ectothelium of the tail is composed of polygonal flat cells, lying side by side. Beneath these is an adhesive cuticle. Between these two epidermal layers are numerous trabeculae, leaving a canal free on both sides of the chorda through which the blood circulates. The blood also enters between the trabeculae. No unicellular glands are found in the tail of the *Oikopleura*.

This genus is widely distributed. Some of the species are found in the surface waters of San Diego region all the year around, being very abundant during the winter months but rare in the summer. Up to date nine species of *Oikopleura* have been taken in this region.

Oikopleura dioica Fol (1872)

Diagnostic characters.—Trunk stout; its dorsal line almost straight but dropping suddenly near the anterior end to form a narrow snout.

The mouth opens upward and has a long underlip (fig. 126, *v.l.*). The endostyle is of medium length. Its posterior end almost touches the inner branchial openings. The two para-endostylar glands, one on each side of the anterior end of the endostyle, are small and almost spherical (fig. 126, *b. gl.*). The otocyst is prominent. The branchial tubes are long and both their internal and external openings are circular (figs. 126, 128). The oikoplast epithelium extends posteriad as far as the anterior half of the alimentary canal. The cells of the lateral oikoplast zone are large (fig. 126, *oik.*). Eisen's oikoplast is large, consisting of four large cells.



Figs. 125–129. *Oikopleura dioica.*

Fig. 125. View of left side of trunk. × 40. Fig. 126. View of right side of trunk of a male animal. × 160. Fig. 127. Dorsal view of tail. × 80. Fig. 128. View of left side of a female animal. × 160. Fig. 129. View of left side of the alimentary canal. × 160. Abbreviations: *a.*, anus; *b.gl.*, buccal or para-endostylar glands; *br.*, branchial openings; *br.t.*, branchial tubes; *end.*, endostyle; *int.*, intestine; *l.s.*, left stomach; *m.*, mouth; *oik.*, oikoplast zone; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; r.*s.*, right stomach; *s.*, stomach; *sch.*, subchordal cells; *t.*, testis; *v.l.*, ventral lip.

The oesophagus opens into the left stomach at the posterior-dorsal corner (fig. 129). The walls of the oesophagus gradually unite with the walls of the stomach without producing any diverticulum (figs. 128, 129). The left stomach is broad, short, and somewhat angular (fig. 127, *l.s.*). The walls of the stomach are composed of large cells.

The right stomach is larger than the left and is divided into an anterior and a posterior portion. The short intestine arises from about the middle of the right stomach. Its lower side joins the posterior end of the rectum (fig. 129).

The gonad when fully developed is large and broadly cup-shaped, its concavity being applied to the digestive tract. Neither testis nor ovary extends over the lateral surface of the alimentary canal, but is limited to the posterior portion of the right stomach. Ovary and testis are in different individuals. This is the only species of *Copelata* known in which this sexual condition exists, all other species being hermaphroditic. The musculature of the tail is comparatively narrow, and on its right side are imbedded two subchordal cells, characteristic of the species.

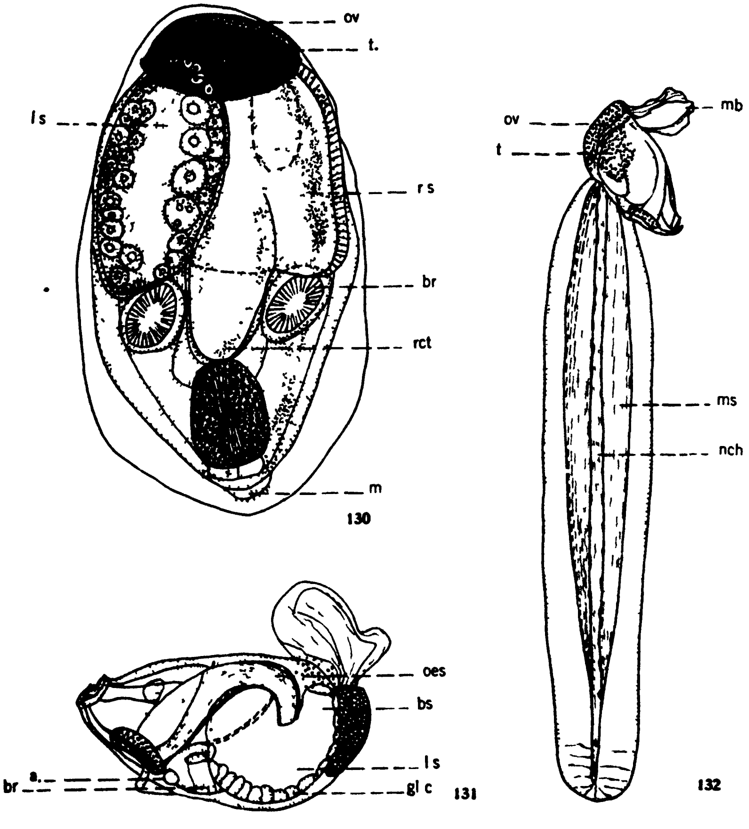
The length of the trunk of a mature *Oikopleura dioica* varies from less than 0.5 mm. to over 1 mm. The dorsoventral width of the trunk is about one-half of its length. The length of the tail is about 4 times the length of the trunk. Its width is about 0.17 of its own length.

Oikopleura dioica is one of the most common species of this region. It is found here in the surface waters practically all year at a temperature varying from 11°5–22°6 C. It is less abundant during the warm summer months, May–August, the numbers varying from 1 to 50 per catch during 1925, and at times being entirely absent from the surface waters. During the colder months, August–May, 1925, the numbers varied from 5–400 individuals per catch. The numbers may still be high in the daily hauls (2 to 300) late in April and in May when all other *Copelata* have practically disappeared from the surface waters except *Oikopleura longicauda* and *Oikopleura fusiformis*, which sometimes appear simultaneously with *Oikopleura dioica.*

The species is very widely distributed and has been found in temperatures from 3°2–29°5 C., and in salinities 11.4 and 36.7 per cent (Lohmann, 1896). *Oikopleura dioica* is a species of the warm waters and of mixed areas but does not frequent the Arctic zones. Lohmann did not find any *Oikopleura dioica* in the Irming Sea, the Greenland stream, nor in the streams of Labrador. It has been found in the North Sea, near the Hebrides Islands, in the Mediterranean Sea, in the Straits of Gibraltar, near Madeira Island, off the west coast of Africa, in the Indian Ocean, and in the Pacific Ocean near the coast of Chili. It has been found in the brackish water at the mouth of the Amazon River and in the Baltic Sea (Lohmann, 1896). In the Japanese waters it is one of the most common species the year round (Aida, 1907). It occurred in the Baltic Sea only during the months from May to December, and in the North Sea, from March to December, while in the warmer waters it occurs the year round, seemingly preferring the near shore waters to the high seas.

Oikopleura longicauda Vogt (1854)

Diagnostic characters.—Body short and stout; Eisen's oikoplast and para-endostylar gland absent; mouth large; endostyle broad and long; branchial openings close to endostyle and rectum; left stomach large, with a prominent blind sac; ovary single; testis paired; tail with wide musculature and without subchordal cells.



Figs. 130–132. *Oikopleura longicauda.*

Fig. 130. Ventral view. × 160. Fig. 131. View of left side. × 80. Fig. 132. The entire animal showing right side of trunk and ventral view of tail. Abbreviations: *a.*, anus; *br.*, branchial openings; *bs.*, blind sac; *gl.c.*, glandular cells; *l.s.*, left stomach; *m.*, mouth; *mb.*, membrane; *ms.*, musculature; *nch.*, notochord; *oes.*, oesophagus; *ov.*, ovary; *rct.*, rectum; *r.s.*, right stomach; *t.*, testis.

Two features by which *Oikopleura longicauda* is easily recognized are its short, thick body and its very wide, long tail (fig. 132). Another feature characteristic of this species alone, is the duplicate membranous structure, one on the ventral, the other on the dorsal side. The dorsal membrane extends anteriad like a transparent veil, sometimes even reaching to the extreme anterior end of the body and overhanging the mouth, or it may be seen only as a rudimentary fold. This structure is similar to the hood of the *Fritillaria* but unlike the hood it does not remain in its original position, and is easily torn off, either partially or completely. In the former case it remains attached to the body only by its posterior edge, while in the latter it slips entirely off the body and floats on the water like a transparent veil (fig. 131, *mb.*). The ventral membrane is very much smaller. No homologous membrane is known in other *Oikopleura*. A complete house is described by Fol (1872) as a spherical structure covering the entire animal, including the tail. No house as big as that described by Fol has been noticed on the *Oikopleura* of this region. Eisen's oikoplast and the para-endostylar gland are absent. The oikoplast epithelium covers the anterior part of the trunk including the anterior half of the alimentary canal (see Lohmann, 1896).

The mouth is large and nearly on the dorsal surface of the trunk. It has a long ciliated ventral lip (figs. 131, 132). The ventral lip has large cells with long cilia on the middle part. Cilia are also found on the dorsal ridge of the mouth. They are more abundant but shorter than those of the ventral lip, and the cilia-bearing cells are smaller. The endostyle is broad and long with its posterior end reaching to the rectum and to the branchial openings. The latter are round and large, but short, one on each side of the rectum.

The stomach consists of two equal-sized lobes (fig. 130). These are connected to each other by their whole length. The left lobe has a large sac ascending upward along the posterior side of the oesophagus, thus forming a deep notch with the rest of the lobe, in which the oesophagus enters. This arrangement gives the left stomach a kidney-shaped appearance (fig. 131, *l.s.*). At the base of the left stomach and partly encircling it along the margin are large glandular cells (fig. 131, *gl. c.*). The right stomach is uniform in shape. The rectum is short and broad with a large anal papilla (fig. 130, *r.s.*, *rct.*). The intestine is also short.

The reproductive glands consist of a single ovary occupying the extreme posterior portion of the trunk, and a paired testis which, when fully developed, extends over the lateral side of the alimentary canal partly covering it (figs. 130, 131). Its posterior part, lying posterior to the alimentary canal is divided into numerous lobes (see Lohmann, 1896).

The tail is long and broad, with a rounded termination. Its wide musculature diminishes gradually toward the distal end and almost disappears short of the end of the notochord (fig. 132). The cells are conspicuous along the chordal walls. The width of the tail is about 0.2 of its length. The greatest width of the musculature is about 0.67 of the entire width. The length of the tail is 4.5 times the length of the trunk.

The trunk of the largest specimens is over 1 mm. in length, but different individuals vary greatly in size.

Next to *Oikopleura dioica*, *O. longicauda* is one of the species that is most abundantly represented in this region. In the warm summer months when its numbers in the surface catches have greatly diminished, the species is still found in abundance in depths of 50 to 100 m.

Frequently when taken from such depths, the animals have a blue or purple color, while those taken from the surface during the colder season are not thus colored.

During the months of August to April the species is found here daily in the surface waters, 5–300 individuals per catch. During the warm summer months (May–August) they cease to be constant visitors in the surface waters, although they may be quite frequently found there in small numbers (0–5 per catch).

According to the observations of Lohmann (1896) *Oikopleura longicauda* is one of the dominating species of the warm areas of all oceans and has the widest distribution of all Copelata, excepting *O. dioica. O. longicauda* was found as far as 60° north latitude in the Gulf stream. It is very abundant in the Atlantic Ocean and in the Mediterranean Sea, where it constitutes about 50 per cent of all Copelata material; and along the entire west coast of Africa where it constitutes 58 per cent of the Copelata material. In the Indian Ocean it was found in the Straits of Malacca, in the Bay of Bengal, and south of it. In the Pacific Ocean it was found near the coast of Chile. In the waters of Japan it is usually found during the warm season of the year and when the south winds prevail, while during the prevalent north winds *Oikopleura longicauda* is very rare (Aida, 1907).

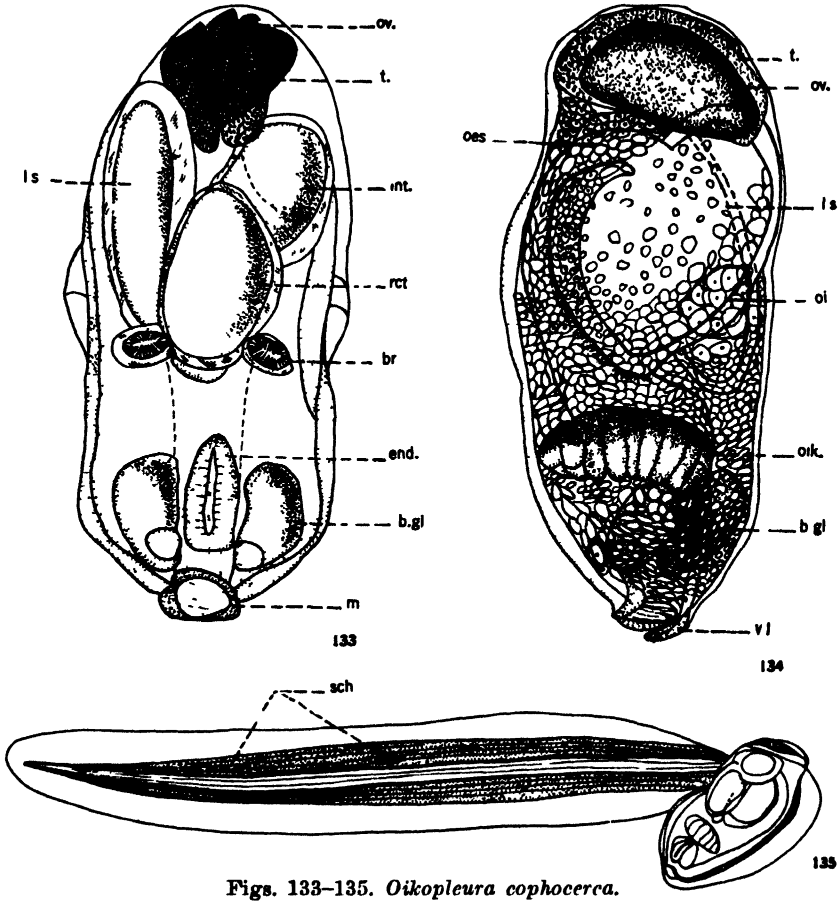
It is interesting to know that in the cold regions, or in the mixed waters as Lohmann calls them, the species is more prevalent during the warm season of the year, while in the warm waters the reverse is true. It is known to be abundant in the Harbor of Messina, November to May (Fol, 1873); in the Indian Ocean in the Canal of Zanzibar, August to March (Lohmann, 1896); in Japan in summer (Aida, 1907); in the Gulf stream at 60° north latitude in July. In this region as already stated, it occurs more regularly and in greater abundance during the winter season August to April at temperature 11°5–17° C., but occasionally it may also be found at a temperature of 23° C.

Oikopleura longicauda is a eurytherm species, a fact which probably accounts for its wide distribution. It has been found in various temperatures from 11°2 to 29°7 C., and in salinities of 37–12.8 per cent. In the Amazon River it was found living in waters that had a salinity as low as 1 per cent (Lohmann, 1896).

Oikopleura cophocerca Gegenbaur (1885)

Diagnostic characters.—Trunk long drawn, its dorsal line slightly curved; oikoplast epithelium extending beyond the alimentary canal, partly covering the reproductive glands; Eisen's oikoplast present; mouth turned upward; endostyle slender; para-endostylar glands very large, as large as the endostyle itself (fig. 133. *b. gl.*); left stomach angular, large; ovary and testis both paired; tail musculature wide, harboring rows of subchordal cells (fig. 135).

Oikopleura cophocerca may be very easily distinguished from any of the other species by its long drawn trunk whose dorsal line is very slightly arched, almost straight, until it reaches the region of the large oikoplast cells where the line drops suddenly toward the mouth (figs. 133, 134). The oikoplast epithelium extends far posteriad, covering part of the reproductive glands on the dorsal surface (fig. 134). A group of large oikoplast cells, Eisen's oikoplast, is found on both sides (fig. 134, *oi.*). The oval-shaped groups of elongated cells comprising the oikoplast zone are very large, situated one on each side of the dorsal line, running transversely (fig. 134, *oik.*).



Figs. 133–135. *Oikopleura cophocerca.*

Fig. 133. Ventral view of trunk. × 80. Fig. 134. View of left side of trunk. × 80. Fig. 135. View of right side of the whole animal. × 20. Abbreviations: *b.gl.*, paraendostylar glands; *br.*, branchial openings; *end.*, endostyle; *int.*, intestine; *l.s.*, left stomach; *m.*, mouth; *oi.*, Eisens oikoplast; *oik.*, oikoplast zone; *ov.*, ovary; *rct.*, rectum; *sch.*, subchordal cells; *t.*, testis, *v.l.*, ventral lip.

The mouth is terminal, slightly turned dorsad, with a long strongly ciliated ventral lip (figs. 133, 134, *m.*, *v.l.*). The endostyle is long and slender (fig. 133, *end.*). The para-endostylar glands (also known as buccal glands) are unusually large. They are kidney-shaped, lying one on each side of the endostyle, each one larger than the endostyle itself (figs. 133, 134, *b. gl.*). The small external branchial openings are located far posterior on each side of the rectum. The oesophagus makes a sharp curve at its posterior end, passing by the stomach and then entering it at the dorsoposterior side (fig. 134, *oes.*). The two lobes of the stomach are connected almost the entire length. The left lobe of the stomach is large, somewhat angular when seen from the lateral view, and has a blind sac at its upper end (fig. 134, *l.s.*). The intestine is short; and the rectum large (fig. 133, *int.*, *rct.*). The reproductive organs consist of paired testes between which are wedged the paired ovaries (figs. 133, 134, *t.*, *ov.*).

The tail is long with a musculature of medium width. On the right side of the chorda are imbedded in the musculature a group of subchordal cells. The arrangement of these cells is characteristic of this species and makes its identification easy (fig. 135, *sch.*).

Oikopleura cophocerca is a large species, although the individuals vary greatly in size. The trunk of the largest specimen measures 3.8 mm. in length. The width of the body is more than 0.33 of its length. The length of the tail is about 4 times the length of the trunk. The width of the tail is about 0.7 of its length. The musculature of the tail in its widest part occupies about 0.5 the entire width of the tail.

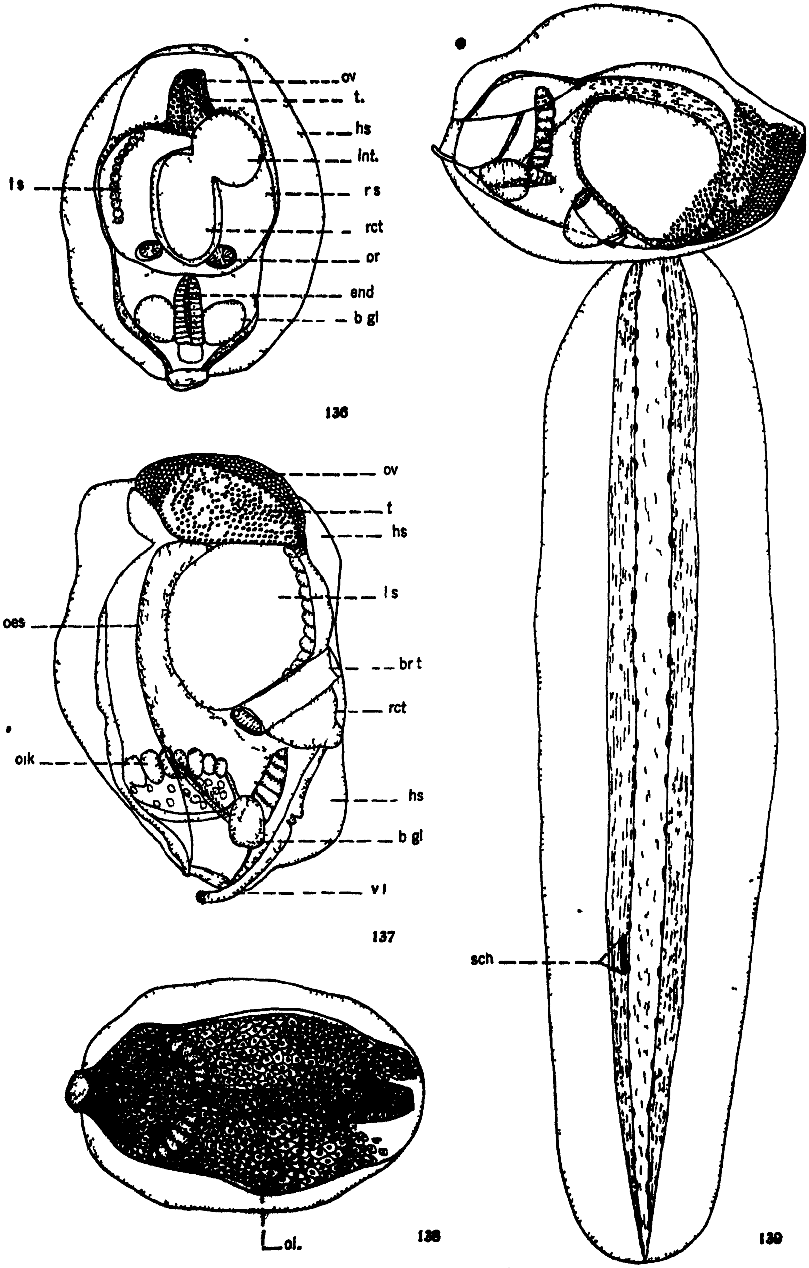
The species is rarely found in the surface waters of this region. About 10 to 15 individuals have been taken from the surface waters at the pier during the winter months, November to March, at temperatures 13°–15° C. Occasionally a few specimens were taken in depths of 50 to 150 meters during June and July, 1920. It has not been observed here in large numbers at any time. The greatest number has been three per catch.

Fol (1872) found it to be a not rare visitor in the Harbor of Messina during November, 1869, and March and April, 1870. Lohmann (1896) found it in all warm-water areas, being represented in the Indian Ocean, in the Atlantic Ocean, and in the Mediterranean Sea. Gegenbaur (1885) found it to be one of the predominating species in the waters of Messina Harbor where it occurred during the winter months, sometimes in great numbers (150–750 individuals per catch). Aida (1907) does not mention it in his report on the Copelata from the Japanese waters. According to Lohmann the species has been found in surface temperatures of 17°6–28° C., and has been taken from depths of 100–200 m. at a temperature of 11°–20°2 C. It was not found below the depth of 200 meters.

Oikopleura rufescens Fol (1872)

Diagnostic characters.—Dorsal line of trunk strongly curved; oikoplast epithelium covers the alimentary canal; mouth terminal; para-endostylar glands spherical; branchial tubes long; left stomach large, without a blind sac; intestine short; ovary single; testis paired; tail long, with narrow musculature and one subchordal cell.

Oikopleura rufescens is a species that may be very easily distinguished from other species by its strongly curved dorsal line (fig. 137) which is raised high in the center over the oesophagus, dropping suddenly toward both ends, giving the animal a typical appearance so



Figs. 136–139. ***Oikopleura rufescens.***

Fig. 136. Ventral view. × 160. Fig. 137. View of left side of trunk. × 160. Fig. 138. Dorsal view. × 160. Fig. 139. The whole animal showing left side of trunk and ventral view of tail. × 40. Abbreviations: *b.gl.*, para-endostylar glands; *br.*, branchial openings; *br.t.*, branchial tubes; *end.*, endostyle; *hs.*, house; *int.*, intestine; *l.s.*, left stomach; *ov.*, ovary; *rct.*, rectum; *r.s.*, right stomach; *t.*, testis; *v.l.*, ventral lip.

that after having once seen an *Oikopleura rufescens* the species is always recognized, even under a low magnification, and without the study of any detailed characteristics. The house is described by Fol (1872) as being very large, oval-shaped, and pointed at one end. All that could be observed in the species of this region is a very rudimentary house (fig. 137, *hs.*). The oikoplast epithelium extends far posteriad covering the digestive organs and a great part of the reproductive organs (fig. 138). Eisen's oikoplast and the oval oikoplast zones are well developed although the latter are comparatively smaller than those of *Oikopleura cophocerca* (figs. 137, 138, *oi.*, *oik.*). The mouth is terminal, turned slightly upward and has a long underlip (fig. 137, *v.l.*). The endostyle is narrow. The para-endostyle glands are nearly spherical (figs. 136, 137, *end.*, *b.gl.*). The branchial tubes are long (fig. 137, *br.t.*), and the small external branchial openings are situated far posteriad (fig. 136, *br.*).

The left stomach is large and almost spherical, with a row of large cells extending anterioposteriad (fig. 137, *l.s.*). From the right stomach arises the short intestine leading into the large rectum (fig. 136, *int.*). Fol (1872) found that the walls of the stomach and the rectum of *O. rufescens* were vividly colored brown, violet, or red and that each cell had a mass of bright pigment. No special colorings have been discovered here in connection with the species. The reproductive glands consist of a paired testis and a single ovary wedged in between the testes (figs. 136, 138, *t.*, *ov.*).

The tail is long and wide, with comparatively narrow musculature. A large elongated subchordal gland cell is near the distal end on the right side of the chorda (fig. 139, *sch.*). This peculiar gland cell is characteristic of this species only and facilitates the identification of the species.

Large specimens measure about 1.5 mm. in length. The width of the trunk is about 0.67 of its length. The length of the tail is about 2.6 times the length of the trunk. Its width is 0.25 of the length.

The species is not common in this region. It appears only occasionally and then in very limited numbers. A few individuals were taken in the surface hauls at the pier on the following dates:

Oikopleura rufescens

Date	Temperature	Number
February 1, 1919	12°9	2
February 2, 1919	12°9	1
March 20, 1919	13°9	1
December 3, 1920	15°6	2
January 10, 1921	13°22	1
March 17, 1921	14°72	1
March 18, 1921	15°8	1

Lohmann (1896) found *Oikopleura rufescens* well represented in all warm waters, extending as far north as to the Florida stream and the Gulf stream. It is considered one of the most common species next to *longicauda*, *fusiformis*, and *cophocerca*. The species was very abundant in the Strait of Malacca, in the Indian Ocean. In the waters off the west coast of Africa it was very scarce, and in the Mediterranean Sea but poorly represented. After several months of collecting in the Bay of Naples, Lohmann found a single specimen of *Oikopleura rufescens* which was so mutilated that it was doubtful whether the species in question was after all an *O. rufescens*. It could not have been very abundant in the Harbor of Messina for Fol (1872) found only ten specimens during the months of May and June while during the winter of 1869–1870 he did not find a single specimen.

Oikopleura rufescens has been found in surface temperatures of 18°9–29° C. It has also been taken from depths of 200 meters at a temperature of 11° C., but has never been found in depths beyond 200 m. (Lohmann, 1896). On this coast the species is known to occur only occasionally during the winter months at a temperature 12°9–15°8.

Oikopleura fusiformis Fol (1872)

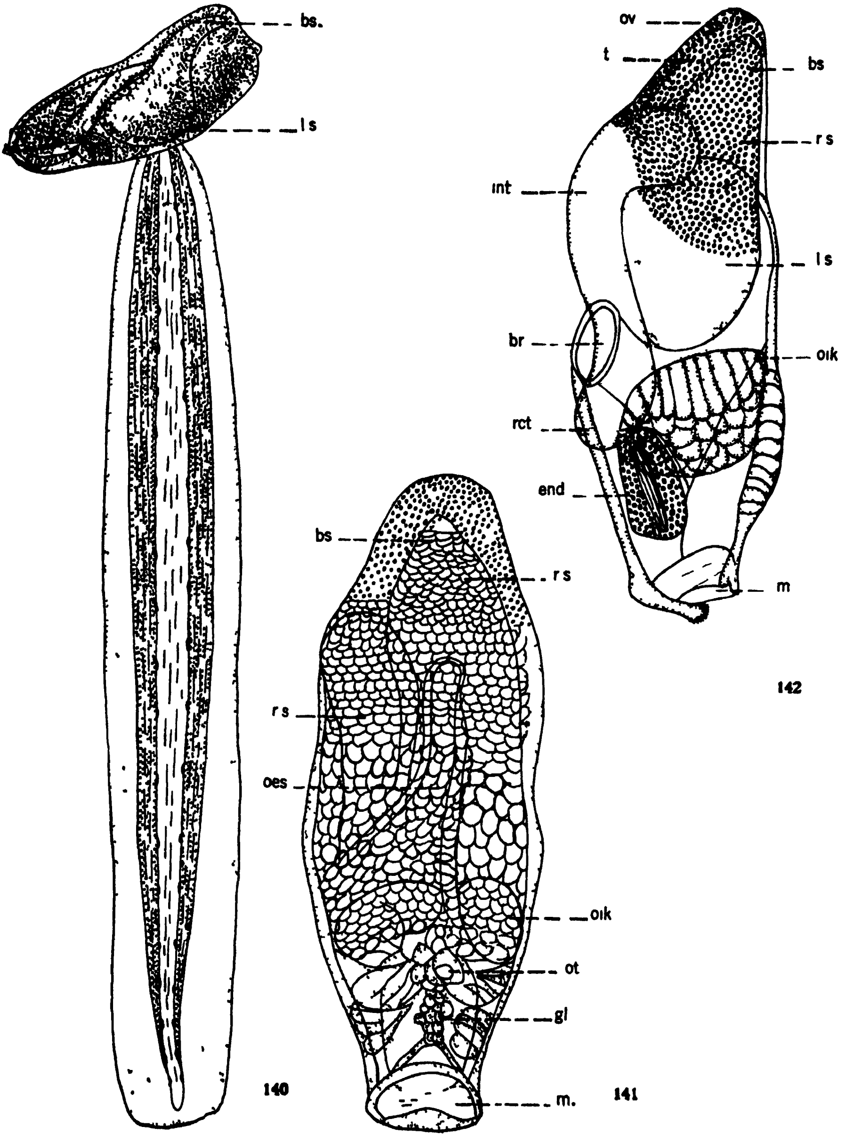
Diagnostic characters.—Trunk long drawn, its dorsal line nearly straight; oikoplast epithelium extends over anterior portion of stomach; mouth almost on the dorsal side of the trunk; endostyle wide; para-endostylar glands wanting; left stomach has a large narrow blind sac (figs. 140, 141, *bs.*); right stomach small; branchial canals short; reproductive glands cover the sides of the alimentary canal; ovary single and flat; testis paired; tail long and without subchordal cells.

Oikopleura fusiformis is another species that may be easily identified by its long drawn trunk with dorsal line nearly straight, its peculiarly shaped left stomach which terminates in a long blind sac, and by its long, straight tail.

The oikoplast epithelium extends over the anterior portion of stomach; the mouth lies almost on the dorsal side of the trunk. The mouth is turned so much upward as to bring it almost to the dorsal surface (fig. 142, *m.*). The endostyle is short and wide and has no para-endostylar glands (fig. 142, *end.*). The branchial tubes are very short. The oesophagus is almost straight and opens at the middle of the dorsal side of the large left stomach (fig. 141). A row of large glandular cells extends along the base of the left stomach, while the upper end of the left stomach is extended in a narrow, long, slightly curved sac (fig. 140, 141, *bs.*). The right stomach is small and is connected with the left by the upper small portion of the latter. The intestine and the rectum are very long, resembling the corresponding organs of *Stegosoma* more than those of *Oikopleura* (fig. 142, *int.*, *rct.*). The testis is paired, long, and angular, and nearly covers both sides of the alimentary canal. The ovary is single and flat (fig. 142, *t.*, *ov.*). In mature individuals the reproductive glands cover the other organs so completely that it is difficult to obtain a view of the digestive system.

The tail is long and the tail musculature narrower than that of *longicauda* and is without subchordal cells (fig. 140). The length of the trunk varies from 0.5 to 1.2 mm. Its width is about 0.33 of its length. The length of the tail is 4 times the length of the trunk. The width of the tail is less than 0.14 of its length.

Next to *Oikopleura longicauda* and *Oikopleura dioica*, *Oikopleura fusiformis* is the most common species of this region. It is seldom found here during the warm season, May to September, but during the winter months, October to April, it is found almost daily in the



Figs. 140–142. *Oikopleura fusiformis*.

Fig. 140. View of right side of the whole animal. × 40. Fig. 141. Dorsal view of trunk. × 80. Fig. 142. View of right side of the body. × 80. Abbreviations: *br.*, branchial openings; *bs.*, blind sacs; *end.*, endostyle; *gl.*, nerve ganglia; *int.*, intestine; *l.s.*, left stomach; *m.*, mouth; *oes.*, oesophagus; *oik.*, oikoplast zone; *ov.*, ovary; *ot.*, otocyst; *rct.*, rectum; *r.s.*, right stomach; *t.*, testis.

surface waters at the pier in limited numbers, 1 to 5 per catch. At times, however, it may be so numerous that 50 to 200 individuals per catch have been taken. In January, 1920, *Oikopleura fusiformis* was for a few successive days the predominating species of Copelata in the surface waters of this region (100–300 individuals per catch). They soon disappeared and have never since been so abundant. No obvious reason suggests itself for such sudden change. The temperature was as usual for this time of year, 13°70–14°30 C.

Oikopleura fusiformis is one of the most common species of Copelata in Japanese waters, being found there practically all the year (Aida, 1907). In the Bay of Messina, the species appeared about the middle of April, 1870, and disappeared by the middle of May. In the spring of 1871 no *O. fusiformis* was found there (Fol, 1872). Lohmann (1896) found the species in all warm areas and as far north as 60° N. latitude, but its distribution was somewhat sporadic. At the estuary of the Amazon River, *Oikopleura fusiformis* was one of the species found nearest the coast. However, it was never found in a salinity below 3 per cent. Its occurrence is known from various parts of the Mediterranean Sea, from the North Sea, from the Atlantic, Indian, and Pacific oceans. It has been found near the coast of Chile, near Japan, and on the coast of California, which indicates that the species has a wide range of distribution in the Pacific Ocean as well as in other seas. The species is seldom found below depths of 200 m. The greatest depth recorded from which it has been taken is 1000 m. in the Mediterranean Sea (Chun, 1888). *Oikopleura fusiformis* has been found in temperatures varying from 9°3–29°5 C. (Lohmann, 1896). On this coast it has been found in temperatures from 12°30–22° C., but the optimum temperature for the species here is 12°30–16° C.

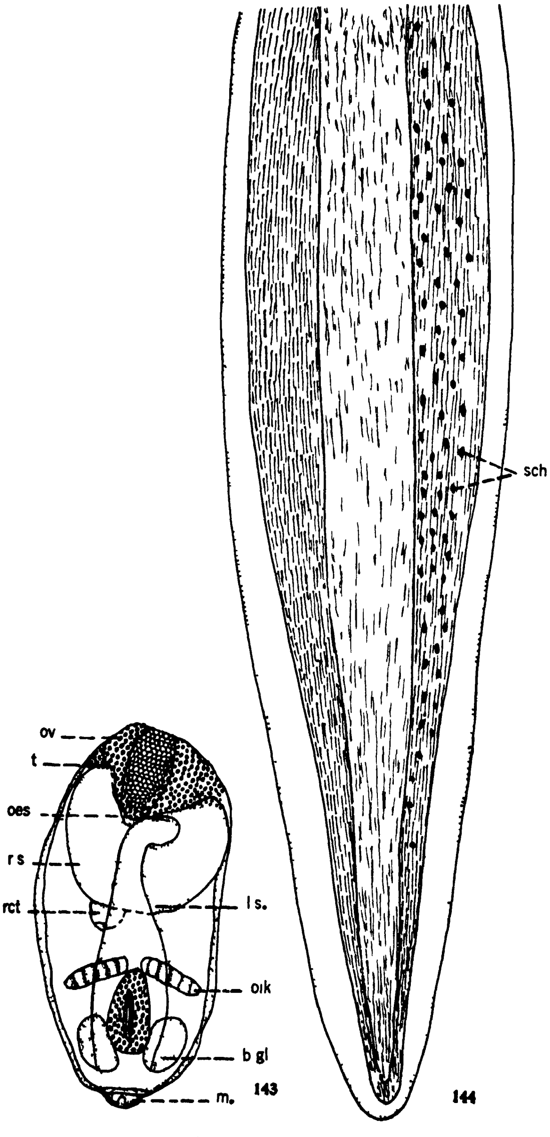
Oikopleura vanhöffeni Lohmann (1896)

Diagnostic characters.—Dorsal line of trunk curved, dropping suddenly toward the mouth region (see Lohmann, 1896); para-endostylar glands elongated; branchial openings widely separated from endostyle; tail with broad musculature and with numerous subchordal cells (fig. 144).

The trunk of *Oikopleura vanhöffeni* is stout with its dorsal line slightly arched, dropping suddenly toward the mouth region (see Lohmann, 1896). The oikoplast epithelium covers nearly the entire body except a small portion of the posterior region of the alimentary canal and the reproductive glands (see Lohmann). The underlip is large and bears a few cilia. The medium-sized endostyle is distinctly broadest at its anterior end (fig. 143); para-endostylar glands elongated. The branchial tubes are widely separated from the endostyle and the small branchial openings are at the base of the stomach. The left stomach has a broad, shallow saculation; the right one a saculation which is narrower and deeper (fig. 143). The intestine is short and the rectum is broad and short. The reproductive glands consist of a paired testis and a single ovary (fig. 143, *t.*, *ov.*).

The long tail has a broad musculature with numerous subchordal cells (fig. 144). Its length is 3.5 times that of the trunk. The length of the trunk is 1 mm.

A single specimen was found in the surface catch after a stormy night. The species is reported by Lohmann as being a well known



Figs. 143–144. *Oikopleura vanhoffeni.* × 160.

Fig. 143. Dorsal view of trunk. Fig. 144. Dorsal view of a portion of tail. Abbreviations: *b.gl.*, paraendostylar ganglia; *l.s.*, left stomach; *m.*, mouth; *oes.*, oesophagus; *oik.*, oikoplast zone; *ov.*, ovary; *rct.*, rectum; *r.s.*, right stomach; *sch.*, subchordal cells; *t.*, testis.

inhabitant of the cold regions only, hence one would hardly expect to find it in this latitude. However, the striking similarity of this specimen with *O. vanhöffeni* as shown by Lohmann, especially as to the numerous subchordal cells of the tail, warrant the conclusion that the specimen found is either *Oikopleura vanhöffeni* or a subspecies of it.

As above stated, a single specimen was found in a surface catch taken at the pier on March 15, 1919, temperature 11°7 C. Lohmann (1896) is of the opinion that *Oikopleura vanhöffeni* is strictly a cold water species which was found by him and Vanhöffen, with one exception, only in boreal regions. It was taken in the Greenland current at a temperature of 3°7 C. In Davis Strait *O. vanhöffeni* was about the only species of *Oikopleura* found during the month of September, while at the beginning of June it was very scarce. It was found near the Shetland Islands, temperature 6°7, and in the North Sea, temperature 5° C.

Oikopleura albicans Leuckart (1853)

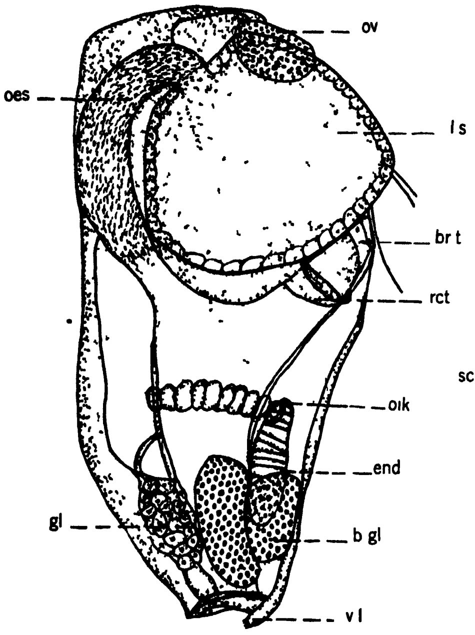
Diagnostic characters.—Trunk long drawn, its dorsal line almost straight; oikoplast epithelium extends to the stomach, covering the anterior portion of it; mouth terminal; branchial openings widely separated from the endostyle; para-endostylar glands large and elongated; endostyle long; tail with a row of circular cells.

Oikopleura albicans has some resemblance to *Oikopleura cophocerca* but differs from the latter in some essential characteristics (see illustrations of Leuckart and Lohmann). The species is easily recognized by its long, slender trunk, whose dorsal line is almost horizontal until near the mouth region, where it takes a sudden drop (fig. 145); by the relative position of the oikoplast zone and the digestive system; and by the peculiarly shaped reproductive organs which in mature state differ from any other species (see Lohmann, 1896, pl. 11, 3).

Eisen's oikoplast and the oval oikoplast zone are well developed. The oikoplast epithelium extends posteriad almost to the cardiac end of the stomach, covering a small portion of its anterodorsal line only, without touching the reproductive glands. The mouth is terminal. The endostyle is long and slender (fig. 145, *end.*). The para-endostylar glands are large and unequal in size (fig. 145, *b. gl.*). The external branchial openings are far posterior on both sides of the rectum. The branchial tubes are very short and their internal openings are far removed from the posterior end of the endostyle. The oesophagus makes a horseshoe-shaped curve before reaching and entering the stomach on its posterodorsal side.

The left stomach is large and angular, the wall containing a row of large cells (fig. 144, *l.s.*). The rectum is short, its tip barely extending below the lower margin of the stomach (fig. 144, *rct.*). The intestine is narrow and short. The right stomach is considerably smaller than the left one. The reproductive glands consist of a single ovary and a paired testis (fig. 146, *ov.*, *t.*). The ovary and testis when in mature state reach an enormous size, and bend over anteriad on the dorsal side, forming a wide horseshoe-shaped mass, without covering the lateral sides of the digestive system. In young animals the reproductive glands form a rudimentary round mass at the posterior end (fig. 145, *ov.*).

The caudal tip is blunt-pointed. The musculature of the tail is broad and in it, on the right side of the chorda, are imbedded numerous large round cells (fig. 146, *sch.*).



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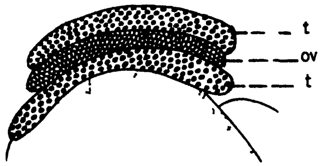

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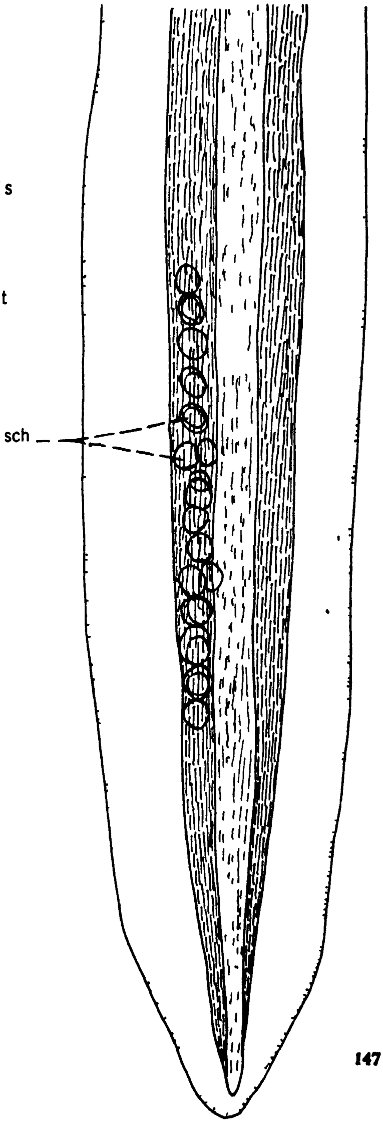

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Figs. 145–147. *Oikopleura albicans.* × 160.

Fig. 145. View of left side of trunk. Fig. 146. Side view of reproductive glands. Fig. 147. Dorsal view of tail. Abbreviations: *b.gl.*, paraendostylar glands; *br.t.*, branchial tubes; *gl.*, nerve ganglia; *l.s.*, left stomach; *oes.*, oesophagus; *ov.*, ovary; *sch.*, subchordal cells; *t.*, testis; *v.l.*, ventral lip.

The length of the trunk is about 1.5–2.5 mm. Its width is 0.5 of the length. The length of the tail is about 5 times the length of the trunk.

The specimens from this coast differ slightly from Lohmann's *Oikopleura albicans* as described in his monograph. The shape of the stomach appears more angular in the specimens of this region than in Lohmann's illustrations. The reproductive glands are less voluminous than those figured by Leuckart and Lohmann. However, these differences hardly justify the recognition of new species, especially in view of the few specimens available for comparison. The species appears to be rare in this region. Only occasionally, after heavy storms, a few individuals have been taken, as follows:

Oikopleura albicans

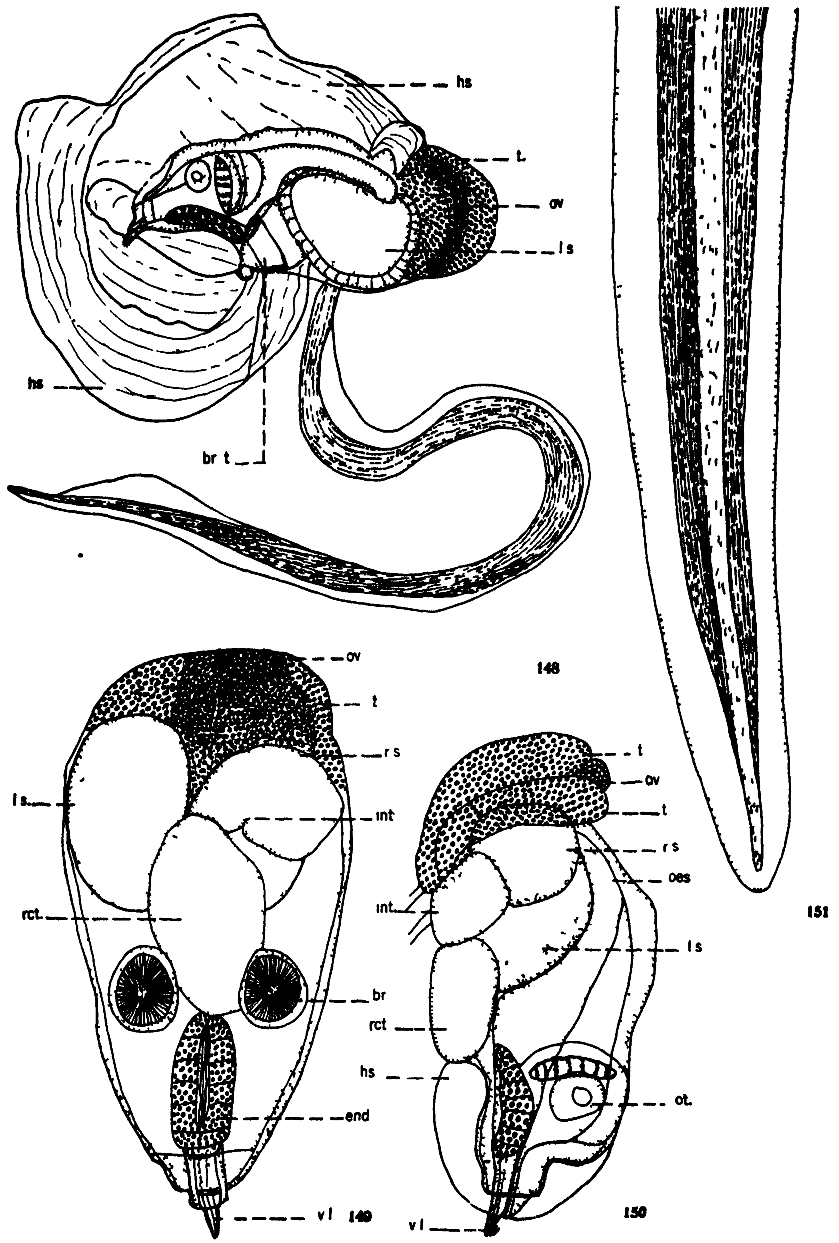
Date	Temperature	Number
January 9, 1919	14°5	1
January 15, 1919	14°5	2
January 26, 1919	15°8	1
March 21, 1919	13°4	3
March 22, 1919	13°4	1
February 3, 1920	11°96	1
March 27, 1920	13°65	1

Lohmann (1896) found the species distributed through the warm-water areas surveyed by him, including the cold islands. However, it was very limited in numbers and not present in all hauls. Near the boundaries of the northern equatorial stream and the Guinea stream (September 2) it was more regularly found. In the Mediterranean Sea the species was found near Villafranche, Naples, and near Messina. A few individuals were found in the vicinity of Channel Islands near Jersey. The species is seldom found below a depth of 200 m., although Chun found it one summer near Naples in a depth of 1000 m. *Oikopleura albicans* has been found in temperatures varying from 11°1–27°2 C. and in salinities 33–37.4 per cent. It is not reported to be abundantly represented in any locality. On this coast the range of temperature in which the species was found is 11°6–15°8 C.

Oikopleura californica sp. nov.

Diagnostic characters.—Trunk slender, its dorsal line curved, dropping suddenly toward the mouth region; endostyle long; para-endostylar glands wanting; branchial tubes short; left stomach somewhat pyramidal (fig. 148), the wall containing large cells; right stomach small, spherical; intestine short; rectum large; testis paired, ovary single; tail long with wide musculature but no subchordal cells (fig. 151).

In its general appearance *Oikopleura californica* has some resemblance to *O. dioica*. The trunk is slender, with its dorsal line arched, dropping almost perpendicularly near the anterior end, forming a narrow anterior region (fig. 150). The oikoplast epithelium covers the anterior portion of the trunk, extending as far as the cardiac end of the



Figs. 148–151. *Oikopleura californica* sp. nov.

Fig. 148. View of left side of the whole animal. × 40. Fig. 149. Ventral view of the trunk. × 80. Fig. 150. View of right side of trunk. × 160. Fig. 151. Dorsal view of portion of tail. × 80. Abbreviations: *br.*, branchial openings; *br.t.*, branchial tubes; *end.*, endostyle; *hs.*, house; *int.*, intestines; *l.s.*, left stomach; *oes.*, oesophagus; *ot.*, otocyst; *ov.*, ovary; *rct.*, rectum; *r.s.*, right stomach; *t.*, testis; *v.l.*, ventral lip.

stomach on the dorsal side, sloping down obliquely on both sides, thus leaving the posteroventral portion of the digestive system free. The oikoplast zone is well developed. The underlip is long (fig. 149, *v.l.*). The mouth is terminal. From the mouth posteriad the ventral line makes an inward curvature giving the extreme anterior end a narrow appearance when seen from the lateral view, so that the rectum protrudes beyond the general outline (figs. 148, 150). The endostyle is long and slender, without any para-endostylar glands (fig. 149, *end.*). The branchial tubes are short (fig. 148, *br.t.*), and the external openings large (fig. 149, *br.*). The oesophagus ascends without making any considerable curvature and opens into the posterodorsal corner of the pouch-shaped left stomach (figs. 148, 150, *oes.*, *l.s.*). The left stomach is somewhat pyramidal (fig. 148), the wall containing large cells. The right stomach is spherical, about 0.5 the size of the left one (figs. 149, 150, *r.s.*). The intestine is short (fig. 150, *int.*), and the rectum large with a prominent anal papilus (figs. 149, 150, *rct.*). The reproductive glands consist of a single ovary wedged in between the paired testis (fig. 150, *ov.*,*t.*). The musculature of the tail is comparatively narrow, more than twice the width of the chorda, and has no subchordal cells (fig. 151).

Oikopleura californica has the biggest house of any species found here, although it does not reach a size equal to that of some species described by Fol, which cover the entire animal including the tail.

The size of different individuals varies. The average length of the trunk is about 0.7–1 mm. The width of the trunk is less than 0.5 its length. The length of the tail is about 3 times the length of the trunk. The width of the musculature of the tail is about twice that of the chorda.

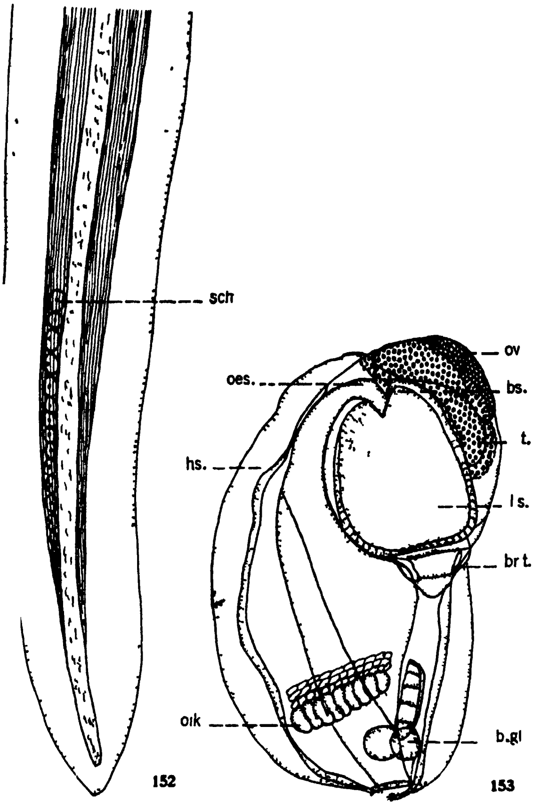
The species has been found here occasionally during the winter season, September to April. Four specimens were found in the surface hauls at the pier on December 23, 1921, temperature 14°3; 2 on March 9, 1922, temperature 12°8 C.

Oikopleura labradoriensis Lohmann (1895)

Diagnostic characters.—Trunk slender; dorsal line strongly raised at the middle, the strong convex curvature toward both ends being regular; lateral and Eisen's oikoplast well developed; Oikoplast epithelium reaches to the stomach, covering a small portion of it; mouth terminal with a long ventral lip; endostyle long and slender with two small para-endostylar glands (fig. 153, *b. gl.*); branchial tubes short; oesophagus makes a sharp bend before entering the stomach; left stomach angular with a blind sac; testis paired; ovary single; tail with a row of large cells.

Oikopleura labradoriensis is well described and illustrated by Lohmann. The high, even curvature of the dorsal line is characteristic. The ventral line of the slender trunk is straight. Eisen's oikoplast is well developed. The oikoplast epithelium extends to the stomach, partly covering the anterodorsal side of it. The mouth is terminal with a long ventral lip, and near the ventral side. Adjacent to the anterior end of the long endostyle are the two medium-sized paraendostylar glands (fig. 153, *b. gl.*). The small branchial openings are widely separated from the endostyle. The branchial tubes are very short (fig. 153, *br.t.*).

The oesophagus turns in a sharp curve near the entrance to the stomach, the entrance being in a conspicuous gastric notch (fig. 153, *oes.*). The left stomach is elongated, quadri-angular in shape with a blind sac, its upper or posterior end being slightly narrower than the lower one and its ventral side longer than the dorsal. The right stomach is oblong, smaller than the left one. The intestine is short and the rectum large with a conspicuous anus.



Figs. 152–153. *Oikopleura labradoriensis.* × 80.

Fig. 152. Ventral view of tail. Fig. 153. View of left side of trunk. Abbreviations: *b.gl.*, paraendostylar ganglia, *br.t.*, branchial tubes; *bs.*, blind sacs; *end.*, endostyle; *hs.*, house; *l.s.*, left stomach; *oes.*, oesophagus; *oik.*, oikoplast zone; *ov.*, ovary; *sch.*, subchordal cells; *t.*, testis.

The reproductive glands consist of paired testes which envelop the single ovary. The gonad mass extends dorsoventrally without covering laterally the alimentary canal (fig. 153, *t.*, *ov.*).

The tail musculature is more than twice the width of the chorda. On the right side of the chorda, imbedded in the musculature is a row of large subchordal cells (fig. 152, *sch.*).

The average length of the trunk is about 0.75–1 mm. Its width is about 0.5 of the length.

The specimens from this coast agree with Lohmann's descriptions of *O. labradoriensis* except that the rod-shaped cells in the house, which Lohmann mentions as one of the distinguishing characteristics of the species, were not discovered in the few specimens available for examination. However, the house or *'Haus Anlage'* is so poorly developed and the number of the individuals examined so small that this characteristic might not have been discovered.

The species occurs in this region during the winter months but only rarely and then in very limited numbers. The few specimens were found on the following dates:

Oikopleura labradoriensis

Date	Temperature	Number
January 24, 1921	12°9	1
February 8, 1921	13°1	5
January 21, 1922	12°8	11
March 21, 1922	12°6	2

Oikopleura labradoriensis is classified as a cold water species and has been found in great abundance up to 70° N. Lat. Lohmann (1896) reports its occurrence from the Gulf stream, from the Irming stream, from North Sea, from Davis stream, and from the Labrador stream to the northern limit of the Florida stream. During the months of May to July it was represented all through the North Sea, along 60° N. Lat., and along the west coast of Greenland. It was abundant in the Labrador stream near New Foundland (800–3000 individuals per catch). In the Irming Sea the species was also very numerous, about 500 per catch.

The average temperature of the surface waters in which this species has been found ranges from 0°48–11°6 C. On a few occasions specimens have been found on the border of the Florida stream in a temperature of 20°1 C. (Lohmann). The few specimens on record from this coast were found in temperatures of 12°6–13° C. The salinity in which the species was found fluctuates from 32.08 to 35 per cent.

Genus **Stegosoma** Chun (1887)

The genus is distinguished from the *Oikopleura* by the peculiar arrangement of its digestive organs. The large left stomach is connected to the right by a narrow tube. The oesophagus opens into the connecting portion instead of opening directly into the stomach. The right stomach is narrow and makes a loop with the continuing rectum. Genital glands in mature state are strap-like and extend over the dorsal, ventral, and lateral aspects of the alimentary canal. Tail long with a narrow musculature.

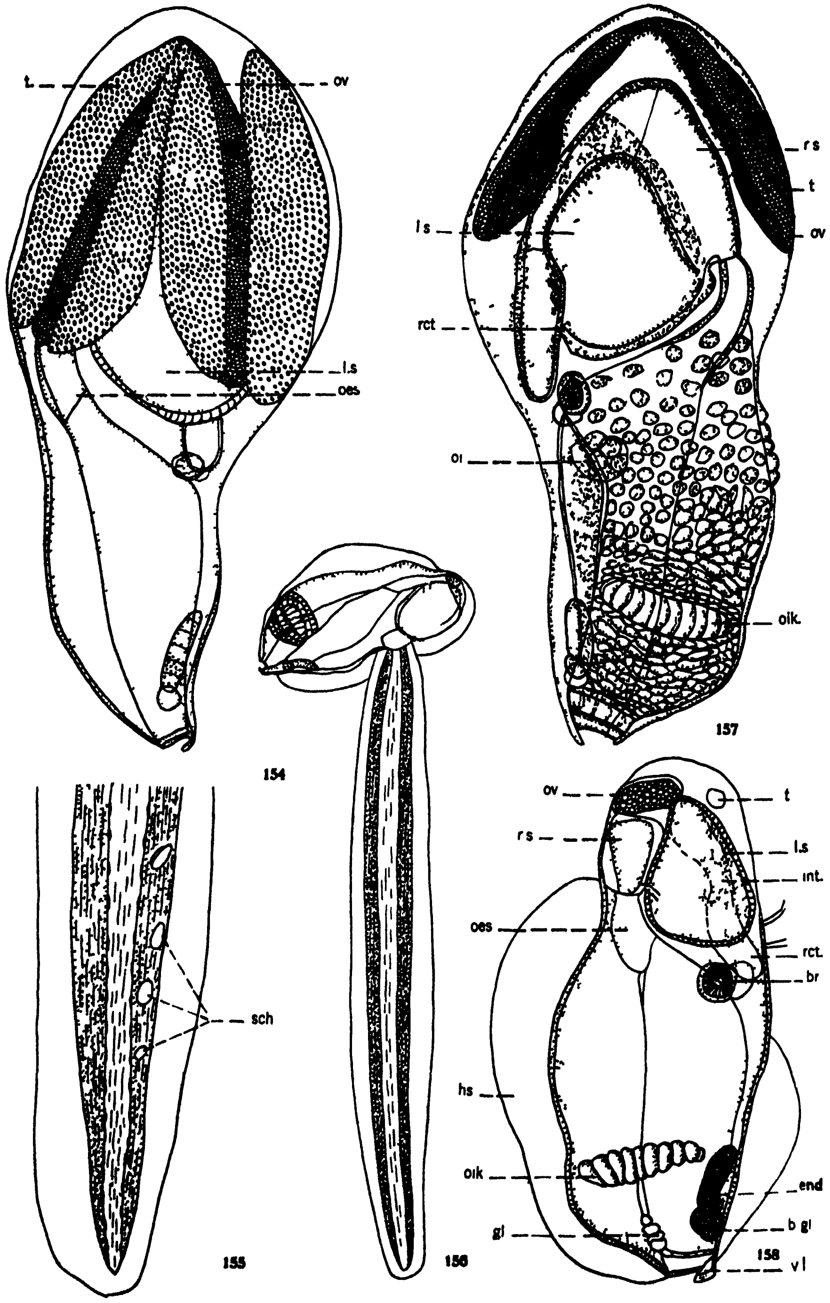
Stegosoma magnum Lghs. (1880)

Diagnostic characters.—Trunk laterally compressed, its dorsal line strongly arched; oikoplast epithelium extends posteriad to the anterior margin of the stomach; oval oikoplast cells and Eisen's oikoplast present; mouth small; endostyle slender; para-endostylar glands round, small; branchial openings far removed from endostyle; oesophagus opens in connecting tube of both lobes of the stomach; left stomach triangular in optical section, large; right stomach long, in continuation of the oesophagus and followed by rectum, forming a loop on the posteroventral surface of the left stomach; reproductive glands in young animals are round masses posteriad of the digestive canal (fig. 158), in older ones extending on the dorsal and ventral and partly over the lateral surfaces (figs. 154, 157); tail with a row of subchordal cells (fig. 155).

Stegosoma magnum is easily identified by its long, laterally compressed trunk whose dorsal line is strongly arched (figs. 156, 157). The oikoplast epithelium is composed of large cells, variously shaped (fig. 157). The oikoplast zone of oval cells is well developed (figs. 157, 158, *oik.*). Eisen's oikoplast is conspicuous (fig. 157, *oi.*). The shape of the trunk varies considerably in older specimens. In young animals it is, for the greater part, uniform in width, with the posterior portion narrower. In mature specimens the reverse is true due to the expansion of the reproductive organs which makes the posterior portion wider than the anterior one. The mouth is terminal and comparatively small. The endostyle is slender (fig. 158, *end.*), and the para-endostylar glands are small and circular (fig. 158, *b.gl.*). The branchial openings are small and far removed from the endostyle (fig. 158, *br.*). The branchial tubes are very short. The pharyngeal cavity is very large.

The oesophagus is short and opens into the connection between the two lobes of the stomach (fig. 158). The wall of the large, pyramidal left stomach contains large glandular cells (fig. 157, *l.s.*). The right stomach is smaller and also pyramidal in shape (figs. 157, 158, *r.s.*). The intestine is long, forms nearly a right angle with the right stomach, and runs forward continuing with little change of form or size, into the rectum (fig. 158, *int.*, *rect.*). The angle thus formed is occupied by the left stomach (figs. 157, 158, *l.s.*). The shape of the left stomach changes considerably in mature specimens. In young animals it is almost round and expanded, while in older, mature specimens it is perfectly flat and angular.

The reproductive glands begin to develop as small globular masses at the posterior end of the trunk (fig. 158, *ov.*, *t.*). As they advance in their development they spread out in layers. When fully developed they take the form of three strands, two of which are the testes, and one (between the others) the ovary (fig. 157, *t.*, *ov.*). Later these break posterior to the middle, making six or more shorter pieces joining one another behind to form a broad V, the limbs of which reach forward on the dorsal, ventral sides (figs. 157, 158, *t.*, *ov.*). With the



Figs. 154–158. *Stegosoma magnum.*

Fig. 154. View of left side of a mature animal. × 80. Fig. 155. Dorsal view of a portion of tail showing the subchordal cells. × 80. Fig. 156. View of the left side of the whole animal. × 10. Fig. 157. View of right side of trunk of a mature animal showing the oikoplast structure. × 80. Fig. 158.

increase in size of the reproductive glands comes a gradual shriveling and probable resorption of the trunk with all the other organs of the animal except the tail. The stomach becomes very much flattened and the whole body becomes narrower (compare figs. 154 and 158).

The tail has a narrow musculature and a row of subchordal cells on the right side of the chorda (fig. 155, *sch.*).

The size of the trunk varies from 1.5 mm. to 3.5 mm. in length. Its width (dorsoventral) in young animals is about 0.5 of the length. The length of the tail is about 3 times the length of the trunk.

Although *Stegosoma magnum* is known as a warm-water species, it is very rare in the surface waters of this region. On February 11, 1920, a specimen was found in a catch taken at the pier, temperature 13°86. On March 19, 1920, two specimens were taken at the same place, at a temperature of 14°17. About twenty other specimens were found, for which no detailed data have been kept.

Aida (1907) records it as occurring in the Japanese waters in midsummer and the early autumn. Lohmann (1896), in his extensive collecting excursion, found the species in all of the warm-water areas, except the cold islands of the south equatorial stream. It was found in the Indian Ocean, in the Canal of Zanzibar, in the Sargasso Sea, and in various stations of the Mediterranean Sea, in the latter frequently in a depth of 800–1300 m. The range of temperature varies from 11°1–27°2 C., and the salinity 34.8–37.4 per cent.

Genus **Megalocercus** Chun (1888)

This genus is closely related to the genus *Stegosoma*. The digestive organs are similar in the two genera. They consist of a large left stomach and a long continuous loop in the right side forming the right stomach, intestine, and rectum. The reproductive glands in both species are also arranged in strands, although differing in form. The shape of the trunk is unique in *Megalocercus*. It is long (3–6 mm.) and uniform in width, the dorsal line dropping suddenly at the anterior end. The oikoplast epithelium extends posteriad over the oesophagus without covering the stomach. The oikoplast zone is very conspicuous. No traces of a house were found. The mouth is small; the endostyle is large and slender. The inner branchial openings are non-ciliated. The branchial tubes are wide but short. The pharynx is large. The oesophagus rises high and makes a sharp angle before entering the stomach on the dorsoposterior side. The reproductive glands in the shape of strands are restricted to the posterior portion of the trunk, forming a trough- or cup-shaped sheet with the cavity turned toward the stomach. The tail is very long.

View of left side of trunk of a young animal. × 40. Abbreviations: *b.gl.*, paraendostylar glands; *br.*, branchial openings; *end.*, endostyle; *gl.*, nerve ganglia; *hs.*, house; *int.*, intestine; *l.s.*, left stomach; *oes.*, oesophagus; *oi.*, Eisen's oikoplast; *oik.*, oikoplast zone; *rct.*, rectum; *r.s.*, right stomach; *s.*, stomach; *sch.*, subchordal cells; *t.*, testis; *v.l.*, ventral lip.

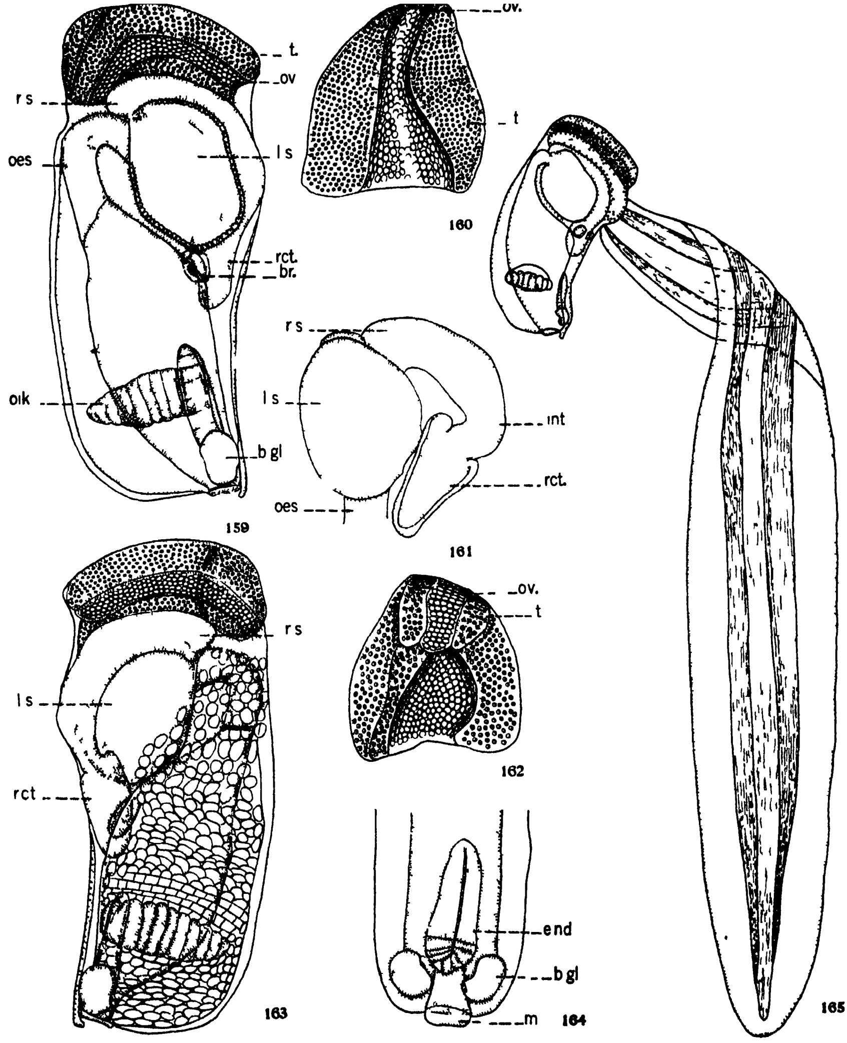
Megalocercus diegensis sp. nov.

Diagnostic characters.—Trunk long, triangular in cross-section, laterally compressed; dorsal line straight, dropping suddenly, almost perpendicularly, at anterior end; oikoplast epithelium extends posteriad covering the oesophagus and a narrow margin of the stomach on the dorsal side (fig. 163); fibrilloplat or oikoplast zone well developed; mouth small; endostyle slender; para-endostylar glands prominent; branchial tubes wide and short, internal openings without cilia; oesophagus turns in a sharp angle before entering the stomach; alimentary system consists of large left stomach, long right stomach, intestine, and prominent pyramidal rectum; testis paired; ovary single; tail long, elliptical, rounded at the end.

The trunk of *Megalocercus diegensis* instead of being uniformly laterally compressed, is triangular in shape with the ventral side of the trunk forming the base of the triangle, which is narrower than either of the lateral sides. A trough-like depression extends lengthwise along the ventral side caused by the two protruding edges (fig. 164). The dorsal line of the trunk is nearly straight and parallel with the ventral to the anterior end where it drops almost perpendicularly, thus giving the animal a uniform width (figs. 159, 163). The oikoplast epithelium extends posteriad covering the branchial region and a narrow dorsal margin of the stomach (fig. 163). The oikoplast zone is very prominent. The mouth is very small with a short underlip. It is terminal and situated far toward the ventral side. The endostyle is long, broadest anteriorly, and rounded at both ends (fig. 164, *end.*). The para-endostylar glands are kidney-shaped (fig. 164, *b.gl.*). The oesophagus, typical of the genus, makes a sharp turn just before entering the stomach on the right side of its dorsoposterior corner (figs. 159, 161, *oes.*). The left stomach is somewhat irregularly elongated anteroposteriorly, its walls containing large cells (figs. 159, 161, *l.s.*). The right stomach is narrow and long, winding along the right ventral side of the left stomach (figs. 161, 163). It is connected with the left by a small middle piece at the entrance of the oesophagus. A short intestine leads from the lower end of the stomach into the large pyramidal rectum (fig. 161, *int.*, *rct.*). The external branchial openings are situated on both sides of the rectum (fig. 159, *br.*). The branchial tubes are wide and short, with the internal openings devoid of any cilia, and are far back (fig. 159). The reproductive glands consist of a paired testis and a single ovary wedged in between the former. They form a thin sheet, occupying the whole posterior end, fitting cap-wise over the posterior end of the digestive organs. When removed from the bódy they look like a cap with the two sides folded and one end bent over (fig. 162, *t.*, *ov.*). When viewed from outside the mass is rounded with all sides turning under (fig. 160, *t.*, *ov.*). The tail is very long, elliptical, its end being evenly rounded. The musculature is twice the width of the chorda. It has no subchordal cells (fig. 165).

The length of the trunk is about 3 to 6 mm. Its height (dorsoventral) is more than one-third the length, and the width of the ventral side is a little more than 0.25 of the length. The length of the tail is more than 4 times the length of the trunk.

So far only two specimens of the species have been found here in a surface haul taken at the pier on April 2, 1921, temperature 14°4 C. Only three specimens of the genus have been found in the Bay of Naples in a depth of 0–900 m. (Chun, 1888). *Oikopleura megastoma* described by Aida (1907) certainly belongs to the genus *Megalocercus* which indicates that this genus occurs also in Japanese waters.

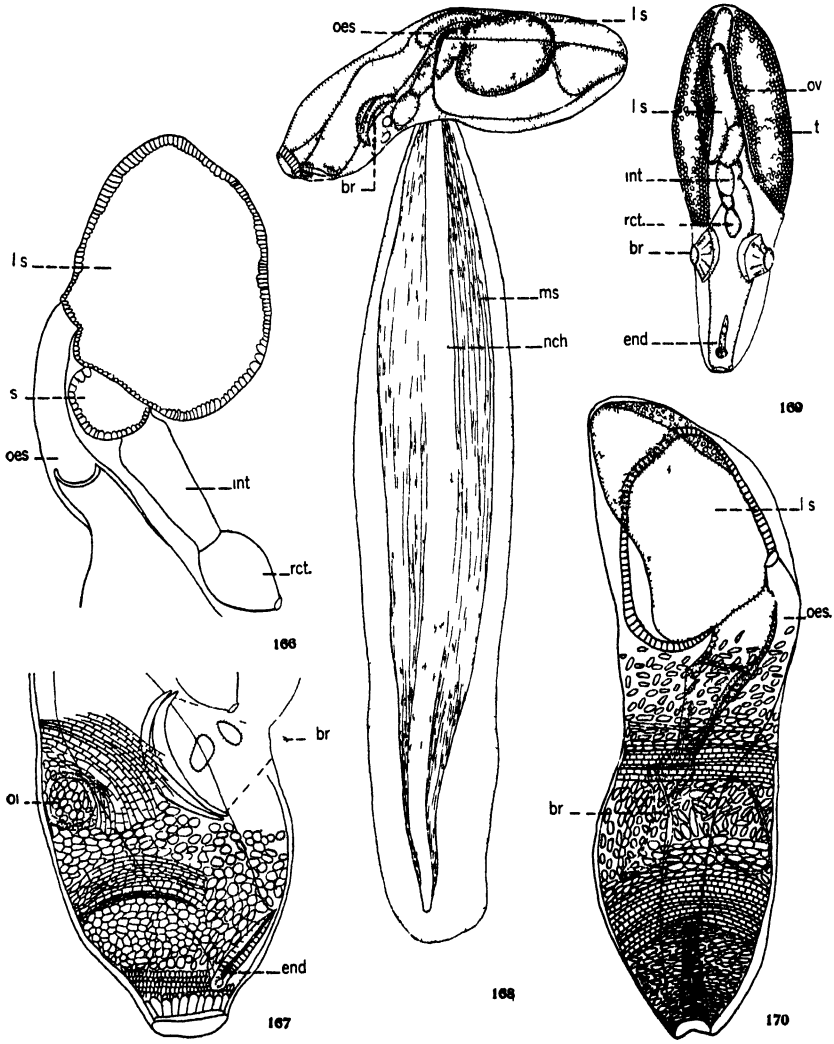


Figs. 159–165. *Megalocercus diegensis* sp. nov.

Fig. 159. View of left side of trunk. × 27. Fig. 160. Outside view of gonads. × 53. Fig. 161. View of left side of digestive system. × 53. Fig. 162. Inside view of the reproductive glands. × 53. Fig. 163. View of right side of trunk (showing the oikoplast cells). × 27. Fig. 164. Ventral view of anterior portion of trunk. × 53. Fig. 165. Left side of the whole animal. × 7. Abbreviations: *b.gl.*, parendostylar glands; *br.*, branchial openings; *end.*, endostyle; *int.*, intestine; *l.s.*, left stomach; *m.*, mouth; *oes.*, oesophagus; *oik.*, oikoplast zone; *ov.*, ovary; *rct.*, rectum; *r.s.*, right stomach; *t.*, testis.

Genus **Althoffia** Lohmann (1896)

Trunk long, *Althoffia*, in this respect, resembling *Stegosoma* more than *Oikopleura*. Stomach a simple pouch. Oesophagus entering stomach on the right side of its anterodorsal region. Intestine arising



Figs. 166–170. *Althoffia pacifica* sp. nov.

Figs. 166. View of left side of alimentary canal. × 27. Fig. 167. View of left side of anterior portion of trunk. × 27. Fig. 168. View of left side of the whole animal. × 7. Fig. 169. Ventral view of trunk. × 7. Fig. 170. Dorso-lateral view of trunk. × 27. Abbreviations: *br.*, branchial openings; *end.*, endostyle; *int.*, intestine; *ms.*, musculature; *nch.*, notochord; *oes.*, oesophagus; *oi.*, Eisen's oikoplast; *ov.*, ovary; *rct.*, rectum; *l.s.*, left stomach; *r.s.*, right stomach; *s.*, stomach; *t.*, testis.

at anterior end and in front of entrance of oesophagus, leading into a pear-shaped rectum. Reproductive glands extending on both sides of stomach in form of a pair of folded, double-layered sheets with the fold ventrad and the loose edges dorsad.

Althoffia pacifica sp. nov.

Diagnostic characters.—Trunk long, laterally compressed; Eisen's oikoplast well developed; oikoplast epithelium extends to the ventral margin of the stomach; mouth small, endostyle narrow; para-endostylar glands wanting; external branchial openings small; interior very large, devoid of cilia; oesophagus enters stomach on right side of antero-dorsal region; intestine arises below the oesophagus on left side; both oesophagus and intestine bent ventrad; tail long with wide musculature.

Althoffia pacifica differs from *A. tumida* in the shape of the stomach which in the former is broad and somewhat triangular in section, while in *A. tumida* it is elongated and sac-shaped (fig. 166). The shape of the trunk and the tail differ in the two species (see Lohmann, 1896).

A. pacifica is a large species. The trunk is long and narrow and laterally compressed (fig. 168). The oikoplast epithelium extends over the anterior branchial region of the trunk (figs. 167, 170). The mouth is small and surrounded by a row of large elongated oikoplast cells. The endostyle is narrow and slender, wider at its anterior end (figs. 167, 169, *end.*); para-endostylar glands wanting. The external branchial openings are very small, the internal openings are very large, crescent-shaped when seen from side view, and are devoid of cilia (figs. 167, 169, 170, *br.*). The short oesophagus enters the stomach on its dorso-anterior side (fig. 166, *oes.*). The stomach is broad, somewhat pyramidal, similar to that of *Stegasoma magnum,* with its base placed obliquely posteroventrally, the apex lying anterodorsally (figs. 166, 168, 170). Similar to *Stegasoma magnum,* the stomach wall contains large cells (figs. 166, 170, *l.s.*). At the anterior end of the left stomach and to the right side is a rudimentary right stomach with large cells (fig. 166, *s.*). From the small lobular part of the right stomach extends the narrow, long intestine leading into a large, pear-shaped rectum turning ventrad (figs. 166, 169, *int.*, *rct.*). The tip of the rectum extends to the branchial openings (fig. 169). The stomach is round in younger animals, but becomes very much flattened in older ones.

The reproductive glands spread out on both sides like a pair of sheets. These sheets, increasing in size, fold and are doubled with the four edges pointing dorsad and the fold ventrad. The ovaries are usually confined to the dorsal margins but are sometimes spread over a greater area, mingling with the testis (fig. 169, *ov.*, *t.*). With the extreme development of the reproductive glands the rest of the body undergoes a considerable degree of atrophy. The stomach becomes flattened and other parts of the body gradually shrivel up, thus changing greatly the general appearance of the animal.

The tail is long, broad, with a broad musculature, especially in the middle region where it is about three times the width of the chorda (fig. 168). The end of the tail is broadly rounded. No subchordal cells are found.

This is one of the largest species of appendicularians of this region. The length of the trunk is 6–8 mm. The width of the trunk is about 0.33 of its length. The length of the tail is 2.5 times the length of the trunk; its width is 0.2 of the length.

The species is not common in this region. On March 17 and 18, 1920, fifteen specimens were taken in the surface catches at the pier, temperature 14°4 C. Eighteen other specimens have been found for which no data are recorded.

DISCUSSION

In this taxonomic study of the Copelata of the San Diego region, or more strictly, of the surface waters near the pier of Scripps Institution for Biological Research of the University of California, seven genera and forty-eight species are described, thirty of which are new to science. The study is far from being an exhaustive one. New species may still turn up occasionally, and many interesting problems pertaining to the various phases of the life of the animals have not been touched in this work. In addition to the new species described, several species hitherto known only from boreal or from tropical seas have been identified at La Jolla. Such observations suggest that strictly limited areas of distribution for species of the marine plankton are rare, if they exist at all. While it is obvious that some species are predominant in certain areas and apparently absent from others, yet the same species can be carried by currents, upwelling waters, and other agencies to areas where they are least to be expected. This fact makes the study of the distribution of plankton a more complex problem than it appears to superficial observation, as the present continuous study of a small area has revealed.

The Copelata are very widely distributed and have been found in all oceans from the equator to 80° latitude and in depths from 0–3000 meters (Lohmann, 1896). Although most species are not to be found below 200 meters, and usually the Copelata become scarce below that depth, nevertheless, some species have been found in good condition in depths of 800–900 meters. The greatest depth from which *Fritillaria* has been taken was 1300–1500 m., but the three specimens were in such bad condition that they could not be identified. At a depth of 2800–3000 m. the tail of an *Oikopleura* was found (Lohmann).

These two cases, however, can hardly be considered evidence that Copelata actually lived in that depth.

Lohmann (1896) in his extensive survey came to the conclusion that Copelata favored the high seas and that the numbers decreased in direct proportion to the nearness of the shore. That these plankton tunicates are not permanent shore-dwellers is evidenced by the fact that they are not always found in the surface waters near the shore, but occur there only occasionally and at irregular intervals. For this reason it is impossible to predict the appearance of a species at any set time. Some species have been found but once in a number of years. Fol (1872) had similar experiences in the Bay of Messina where he found certain species during a few months, but did not find them again the following year. Other species he found there which were more or less abundantly represented during certain months in the year, usually during the winter and early spring. Aida (1907) speaking of the distribution of Copelata says that even the most common species are not always to be found in the waters of Japan, and that during the winter months they were rarely found in the surface waters. But during the summer months, when the south winds are blowing and the current is sweeping near the coast of Japan, the appendicularians are frequently found near the shores.

On the coast of California the reverse is true. The Copelata are more abundant, both individuals and species, in the surface waters during our winter season. With the exception of *Oikopleura dioica* and *Oikopleura longicauda*, hardly any other species are found here in the surface waters during the summer season. But even these very common species are not found here every day.

During the summer months (June–August) hauls have been taken 5–15 miles offshore from depths of 50–150 m., where the most common species of *Oikopleura* were found in considerable numbers, but none of the rare species of either *Oikopleura* or *Fritillaria* was among them. This seems to indicate that those species do not live permanently anywhere near the shore but are probably carried from the high seas to the coast by favorable winds and currents. This assumption that the Copelata live on the high seas would account for their irregular seasonal distribution.

That these tunicates depend for their distribution upon some agency is beyond question, for with their means of locomotion they could make very little progress, and would have very little choice of direction, being carried along with the moving body of water. Since the circulation of the waters is greatly influenced by the winds, the direction and velocity of the winds are of some importance in distribution of marine plankton. In California there are only two seasons: a dry summer season with prevailing northwesterly winds causing a southwest drift of the surface waters of the Pacific Ocean; and a more or less rainy winter season with the prevailing southwesterly winds and a more irregular movement of the surface waters. With the seasonal changes of direction of the surface waters in general, there should be expected some change in the distribution of plankton organisms.

Besides the general drift of the surface waters, controlled to a greater or lesser extent by the prevailing winds, there is the continuous, slow, northward drift of the cold bottom water from the Antarctic, as well as the three main currents: The Kuro-Siwo (the Japanese Current), flowing south and west; the California Current, flowing southwest; and the Davidson Current, flowing north. Of these three currents the Kuro-Siwo is the farthest off shore and may not have much, if any, direct influence on the coast, but the other two may be regarded as of considerable importance. The California Current is strongest in summer. The narrow Davidson Current flows north bordering the coast and is of variable strength. Probably the southward flowing California Current occasionally touches the coast, due to a shift of the ocean winds. Add to this complex system of currents and the general drift of surface and bottom waters, the upwelling bottom water and its tendency to flow toward the coast, the occasional storms, cyclones, and tides, and one gains some idea of the various means of conveyance and the chances by which organisms may be carried great distances from their original dwelling places. After realizing this it is not surprising to find a species at La Jolla that has previously been reported either from the coast of Chile or from 60°–70° N. latitude. By the natural means of conveyance, species can be carried from any part of the ocean, which accounts for the great number of species found here occasionally. Less than one-tenth of the species described in this paper are found here at all regularly or seem to live near this coast. What happens to the migrant species is not known but undoubtedly they are doomed to perish because of the unfavorable conditions.

Some of the obvious necessities for the existence of Copelata are proper food, temperature, light, and chemical composition of the water. It hardly seems possible that the Copelata would perish here near the shore for lack of food as there is an abundance of microorganisms. However, they would probably suffer from a superabundance of microörganisms, such as *Chaetoceros,* etc., which might clog up the meshwork before their mouths, and cause them to cast off the house with the meshwork, thus rendering them more liable to the attacks of enemies. Among their most common enemies are the Copepoda and some small Medusae. When examining fresh plankton material one often sees a Copepod gradually devouring a Copelata, usually attacking it from the tail, holding and following its victim until the latter has been completely devoured. Medusae attack Copelata in a similar way, although they may sometimes begin with the trunk. Occasionally *Oikopleura* have been found with unusually short, rudimentary tails, which would seem to indicate that sometimes, after being attacked by an enemy, the appendicularians have managed to escape, and their tails have regenerated. Whether the food conditions and the enemies of Copelata near shore are sufficient to account for their small numbers or for the almost complete absence of some species, remains an open question.

Of equal importance with the food conditions seems to be the influence of temperature. There appears to be an optimum temperature within the range of which the animals are more abundant (Essenberg, 1922). According to the reports of Lohmann (1896), Copelata have been found in much higher temperature on the high seas than they have ever been found here. Lohmann states, however, that the Copelata were taken from a vertical column of water from a depth of 0–200 m., so that the actual depth at which the animals were taken is not known. Only the surface temperatures are given. Should the tunicates have come from the lower layers, which probably was the case, the actual temperature in which the animals were found would not be 22°–29° C. but considerably lower. Some species of Copelata are more adaptive to changes of temperature and are able to live in a great range of temperature. Some species are even known to stand a difference of 27° in temperature. It is also interesting to know that it is the latter species that have the widest distribution and among them are our permanent shore dwellers. Probably they are the most widely distributed because of this ability to adapt themselves to the various changes of temperatures and thus to drift with either cold or warm currents, while more delicate species, sensitive to changes of temperature, die in the unfavorable temperature before reaching our shores, or shortly after, especially if they happen to be here during our summer season when the temperature is high.

Light may have some effect on the distribution of Copelata, for they are not often found in depths beyond 200 m. It is also known that the same species that are found in great abundance in the surface waters in some places, during the colder season, are, during the warmer season of the year, found only in deeper layers. Whether it is the effect of light or temperature that causes the migration, or the combined effect of both, cannot be positively stated, but I am inclined to believe that the temperature has the greater influence.

The chemical composition and the salinity of the water here undergo comparatively slight changes. The salinity fluctuates between 33 per cent and 34 per cent. Considering the great changes of salinity (1 per cent to 37 per cent) that some Copelata can stand, this change seems almost negligible.

There may be some question as to the suggested means of migration and the causes of distribution of Copelata. But this much can be said with assurance, that very few species are found here near the shores the year round, and even the most common ones cannot be found every day. The great bulk of the species may arrive by some chance during our winter season, but they evidently find the conditions unfavorable for their existence and perish. The same species does not reappear at the same time each year but may be abundant in one year and completely absent the following year, so that no definite time can be predicted for the recurrence of any species on this coast.

While the work reported is far from being exhaustive, it shows that by a constant, persevering study of the plankton material of a very limited area, one may find as large a variety of species as by making an extensive survey of great oceans. Whether this is due to the exceptionally favorable location for such studies of the Scripps Institution cannot be determined until similar continuous plankton studies are made in other localities on the coast.

LIST OF SPECIES

- Family Kowalevskidae
    - Kowalevskia tenuis Fol
- Family Appendicularidae
    - Subfamily Fritillarinae
        - Appendicularia sicula Fol
        - Fritillaria amygdala sp. nov.
        - Fritillaria angularis sp. nov.
        - Fritillaria artus sp. nov.
        - Fritillaria borealis Lohmann
        - Fritillaria brevicollis sp. nov.
        - Fritillaria campila sp. nov.
        - Fritillaria claudaria sp. nov.
        - Fritillaria clava sp. nov.
        - Fritillaria delicata sp. nov.
        - Fritillaria diafana sp. nov.
        - Fritillaria dispara sp. nov.
        - Fritillaria exilis sp. nov.
        - Fritillaria formica Fol
        - Fritillaria gigas sp. nov.
        - Fritillaria haplostoma Fol
        - Fritillaria inverta sp. nov.
        - Fritillaria juncea sp. nov.
        - Fritillaria limpida sp. nov.
        - Fritillaria lohmanni sp. nov.
        - Fritillaria lucibila sp. nov.
        - Fritillaria macrotrachela sp. nov.
        - Fritillaria nitida sp. nov.
        - Fritillaria pellucida Bush
        - Fritillaria plana sp. nov.
        - Fritillaria pulchrituda sp. nov.
        - Fritillaria ritteri Aida
        - Fritillaria sargassi Lohmann
        - Fritillaria tacita sp. nov.
        - Fritillaria tenebra sp. nov.
        - Fritillaria tereta sp. nov.
        - Fritillaria trigonis sp. nov.
        - Fritillaria truncata sp. nov.
        - Fritillaria velocita sp. nov.
        - Fritillaria venusta Lohmann
    - Subfamily Oikopleurinae
        - Oikopleura albicans Leuckart
        - Oikopleura californica sp. nov.
        - Oikopleura cophocerca Gegenbaur
        - Oikopleura dioica Fol
        - Oikopleura fusiformis Fol
        - Oikopleura labradoriensis Lohmann
        - Oikopleura longicauda Vogt
        - Oikopleura rufescens Fol
        - Oikopleura vanhöffeni Lohmann
        - Stegosoma magnum Langerhaus
        - Megalocercus diegensis sp. nov.
        - Althoffia pacifica sp. nov.

SUMMARY

This paper contains an account of forty-eight species of the Copelata, appendicularians obtained from plankton collected from the Pacific Ocean at or near La Jolla, California. Of these species thirty have not previously been described.

The Copelata of California waters, contrary to the conditions described for the coast of Japan, are more abundant both in species and in individuals during the winter season. Rarely more than two of the common species occur during summer months in the plankton near La Jolla. On the coasts of Japan the maximum number occurs during the summer.

The relative number of species of Copelata known from this coast is much greater, being four times that from the Japanese coast. Three-fourths of the number of species belong to the genus *Fritillaria,* while on the coast of Japan only one-fourth of the species found there belong to this genus.

Of the eighteen species of Copelata previously described, eight are of the group from strictly warm-water, four are from cold-water areas, and five are from mixed areas or are cosmopolitan species, according to Lohmann's classification.

The most eurythermal species are of the widest known distribution and occur on this coast the year around. Species from supposedly restricted areas are never found here during the summer season.

Even during the winter season not all species of Copelata are found here every day, and some may not occur two winters in succession. This suggests that they are not permanent coastal species but are carried by currents and drifted to the shore by irregular ocean winds and by waters moving toward the coast.

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OBSERVATIONS ON GRADUAL DISINTEGRATION AND DEATH OF COPELATA

BY

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BY

CHRISTINE E. ESSENBERG

(Contribution from the Scripps Institution of Oceanography)

It is well known to students of tunicates that members of the genus *Oikopleura* are very delicate and that, when subjected to sudden changes or when specimens are kept in a small dish in the laboratory, they soon turn opaque and die. It is noticeable, in watching *Oikopleurà* under such conditions under the microscope, that death occurs gradually. At first the animal as a whole becomes less active, the flagella around the mouth gradually beat more slowly until they become completely inactive, while other organs of the body are still functioning. Beginning with the anterior end, death advances gradually toward the posterior end. Thus the posterior portion of the trunk lives considerably longer than the anterior portion, although the body may remain intact and keep its original shape for some time.

In many instances, however, the body disintegrates, pieces gradually crumbling away. This process, also, begins at the anterior end of the trunk. The body walls near the mouth and lips disappear first, while the endostyle is still functioning and the branchial cilia are beating vigorously. The endostyle crumbles away next; the branchial cilia cease their activity, and the branchial openings and ducts disappear. One organ after another disintegrates until the animal is reduced to only the digestive system, viz., the oesophagus and the stomach (figs. 2–5), and the tail. This remaining posterior portion of the animal can still live in a drop of water for two to four hours. The cilia of the oesophagus continue beating vigorously, conveying to the stomach such microörganisms as happen to come in their way. The stomach is also carrying on the digestive process, as may be judged from the fact that faecal matter is extruded from the rectum from time to time. If the tail has not been detached from the trunk, the animal, even in this degenerated condition, moves about vigorously for several hours, gradually slowing down. If the tail is carefully removed without injury to it or the trunk, the trunk, deprived of the organ of locomotion, naturally remains stationary, while the tail continues to move about independently with its proximal end first, as if still pushing the body. The tail may live in this condition from one to three hours.

The gradual disintegration of a number of specimens of *Oikopleura* in a drop of water has been watched, under the microscope. Occasionally individuals are found in an observation dish moving about vigorously although the anterior portion of the trunk is gone. In this condition their appearance is so completly changed that to one not familiar with the Copelata they may appear to be animals belonging to an entirely different group. If the trunk is cut into halves, as has been often done by the aid of fine needles, the anterior end ceases to function almost immediately while the posterior end may continue to live for several hours after the operation.

In some Copelata, e.g., the genera *Stegosoma* and *Althoffia* and *Fritillaria pellucida* and other species, the reproductive glands develop to an enormous size. Coincident with this extreme development of the ovaries and testes, the rest of the body usually undergoes a gradual degeneration. Although the digestive organs may change their shape, becoming more flattened, the real degeneration begins with the anterior end and advances posteriorly. For instance, individuals of *Fritillaria pellucida* have been found with all the anterior organs disintegrated, the body wall shriveled up, and the reproductive organs enormously expanded (Essenberg, 1924). Occasionally other species of Copelata are found in a similar condition.

It would be interesting to know whether such specimens of *Oikopleura,* after degenerating to such an extent that half of the trunk has disappeared, can still live and regenerate the lost parts if kept under natural conditions. Laboratory conditions, however, particularly the necessarily small volume of water, are not favorable for even normal individuals to survive very long. Due to their very small size, the *Oikopleura* cannot very well be kept under observation in their natural environment. Even if it were possible to keep the animal by some device in the ocean or in a big tank of water, the necessary handling would disturb it so severely as to make recovery improbable. The extreme delicacy of these tunicates is shown by the fact that, whenever an *Oikopleura* is caught in a net, it throws off its house, so that one is never caught with a house on it. The house is normally replaced within six to ten hours, but it is reasonable to suppose that the secretion of the house would consume a great deal of the energy and strength of the animal and thus reduce the reserve power needed for the growth or regeneration of lost parts. Whether such vital organs as the endostyle, the pharynx, and the branchial ducts can regenerate, has not been ascertained. There is evidence, however, that the tail may be regenerated because specimens of *Oikopleura* have often been found with very short tails, a fact which indicates that the tail had been completely lost and had been regenerated, or that its end had been bitten off by some animal and that later it had been at least partly regenerated. The sieve-like structure guarding the mouth of an *Oikopleura* is frequently thrown off and a new one regenerated to replace it.

No special effort has been made to ascertain the degree of power of regeneration in *Oikopleura*. The observed evidence, however, shows that the animals degenerate and die by degrees and that the anterior end of the trunk disintegrates and dies first. If the anterior end is separated from the posterior one, all the organs in the anterior end stop functioning immediately, while the posterior half can live several hours with all its organs functioning.

The degenerative process here described exhibits the presence of an axial gradient in this chordate not unlike that of the lower invertebrates as described and experimentally demonstrated so fully and frequently by Professor C. M. Child.

INDEX

Univ. Calif. Publ. Zool., vol. 28

Titles of papers and new systematic names are in **boldface**;
synonyms are in *italics*.

Acknowledgments, 30, 67, 127, 155, 173, 183, 252, 294, 363, 366, 400.
Agglutination tests, with cultures of Leishmania and Herpetomonas ctenocephali, 369; of Noguchi, 372.
Albatross, U.S.S., collection of new Dinoflagellata, 205.
Althoffia, 510.
  pacifica, 511.
Amitermes arizonensis, 397.
  californicus, 397.
  confusus, 397.
  tubiformans, 397.
Anoplotermes fumosus, 397.
Antennularia **verticillata**, 171.
Antiserums, immunological reactions with cultures of Leishmania, 36; of Herpetomonas ctenocephali, 368.
Appendicularia sicula, 415.
Arizona termites, 389–397; description of two new species, 393–396.
Beechwood creosote, 358.
Behavior of Endamoeba dysenteriae in Mixed Cultures with Bacteria, 127–154.
Benzyl benzoate, 358.
Bimeria **pulsilla**, 168.
  tenella, 168.
Biological Relationships of Leishmania and Certain Herpetomonads, 365–388.
Blanc, H., on division of Ceratium hirundinella, 30.
Borgert, A., on unipolar splitting of chromosomes in Ceratium, 38; on division in C. tripos and C. fusus, 40, 45, 47.
Blastocystis, 130.
Boeck and Drbohlav, use of glucose in experiments with Endamoeba gingivalis, 101.
Calkins, G. N., cited on longitudinal splitting of chromosomes in Noctiluca, 46.
Calonympha, 293, 301.
Calotermes castanens, 294.
Campanularia **castellata**, 170.
Cat, cestodes found in, 323; host of: Dipylidium trinchesi, D. pasqualei, 331; D. chyzeri, 332; D. oerleyi, D. sexcoronatum, 333.
Causey, D., 1–28, 217–250.
Ceratium hirundinella O.F.M., Mitosis in, with Notes on Nuclear Phenomena in Encysted Forms and the Question of Sexual Reproduction, 29–64.
Ceratium hirundinella, division of, 30, 44; neuromotor system, extranuclear centrosome, paradesmose, cytoplasmic inclusions, 32; interphase of nucleus, 33; prophase, 34; karyosomes, 35; intranuclear rhizoplast, 36; longitudinal splitting of chromosomes, 37, 46; unipolar splitting, 37; formation of metaphase plate, 39; anaphase and telophase, cytoplasm of cyst, nucleus of cyst, 41; chromosomes of cyst nucleus, binucleate encysted forms, 42; evidence for nuclear division in cyst, 43; evidence supporting conjugation, 48; annucleate forms, 50; sexual phenomena, 51.
Ceratium tripos, centriole in karyosome of, 36; transverse division of chromosomes, 40; amitosis, 47.
Cestode Genus Dipylidium from Cats and Dogs, 317.
Cestodes, in dogs, 322; in cats, 323.
Child, H. J., 251–276.
Chilomastix, 290.
Ciliates, significance of granules of neuromotor system, 238.
Clearing Fluid Formulated by Spalteholz, A Useful Modification of a, 357–359.
Columba, sp., host of Dipylidium columbae, 319, 333.
Copelata, material and methods, 400; classification, general morphology, distribution, 401; discussion, 512; list of species, 517; summary, 518.
Copelata from the San Diego Region, 399–521.
Copelata, Observations on Gradual Disintegration and Death of, 523–525.
Coryne **corrugata**, 167.
Cryptolaria pulchella, 172.
Cuvier, primitive duct of, 361, 362.
Cynictis penicillata, host of Dipylidium zschokkei, 319.

Cytoplasm, amoeboid, 253; leucocytic, 257.

Cytoplasmic inclusions, theories as to function of, 1.

Dinophysoidae, and Peridinioidae, convergence between, 205.

Dipylidiinae, subfamily, 318.

Dipylidium, On the Cestode Genus, from Cats and Dogs, 317–356.

Dipylidium, characteristics of genus, 318; fixing, staining, and mounting of material, 320–321; descriptive table of, 331; key to, 336; new species, comparison as a group, 338; comparisons: **crassum** and **longulum**, 339; **compactum, diffusum,** and **gracile**, 340.
  avicola, 319; description and host, 333.
  caninum, type species of genus, 318; detailed description, 324; host, 331.
  chyzeri, 319; description, host, 332.
  columbae, 319; description, host, 333.
  compactum, description, 328; host, 335.
  crassum, description, 330; host, 336.
  diffusum, description, 328; host, 335.
  dongolense, 319; description, host, 334.
  echinorhyncoides, 318; description, host, 331.
  genettae, 318.
  gervaisi, 319; description, host, 332.
  gracile, description, 326; host, 335.
  longulum, description, 329; host, 336.
  monticellii, 318.
  monoöphoron, 319; description, host, 332.
  oerleyi, 319; description, host, 333.
  pasqualei, 318; description, host, 331.
  quinquecoronatum, 319; description, host, 334.
  sexcoronatum, 319; description, 325; host, 333.
  sp. of Kofend, description, host, 335.
  trinchesi, 318; description, host, 331.
  triseriale, 319; description, host, 332.
  walkeri, 320; description, host, 334.
  zschokkei, 319; description, host, 334.

Dog, cestodes found in, 322; host of Dipylidium sexcoronatum, 333.

Drbohlav, and Boeck, use of glucose in experiments with Endamoeba gingivalis, 101.

Duct of Cuvier, 361, 362.

Earliest Blood Vessels of the Mammalian Embryo Studied by Means of the Injection Method, 361–364.

Effects of Certain Drugs and Dyes upon the Growth of Endamoeba gingivalis, 173–182.

Effects of Certain Drugs upon Endamoeba dysenteriae in vitro, 155–166.

Embryo of pig, cardinal veins, duct of Cuvier, 361.

Embryo of rat, injected, 363.

Embryo, Mammalian, the Earliest Blood Vessels of the, Studied by Means of the Injection Method, 361–364.

Emerson, A., collector of Kalotermes, 286; intestinal smears made by, 296.

Endamoeba buccalis, food of, 254.

Endamoeba gingivalis (Gros), The Cultivation of, 65–126.

Endamoeba gingivalis (Gros) Brumpt, Mitochondria and Golgi Bodies in, 1–18.

Endamoeba gingivalis (Gros) in vitro, The Effect of Certain Drugs and Dyes upon the Growth of, 173–182.

Endamoeba gingivalis: in Mixed Bacterial Cultures; in Filtered Saliva; on a Solid Base; with Peritoneal Cells; and with Digestive Secretions, 183–202. **Studies on the Ingestion of Leucocytes, and on Mitosis in,** 251–284.

Endamoeba gingivalis, morphology of motile phase, mitochondria, 4; golgi apparatus and function, 5, 6; fat bodies, 6; amoeboid movement, Brownian movement, 8; spiral organization, 10; attempts at cultivation, 66; nomenclature, 67; relationship to pyorrhoea, 67; culture medium for, egg slants, 68; Locke's solution, Locke-albumen solution, 69; methods of inoculating tubes, 70; hydrogen-ion concentration, determination of, 71; from different cases, 73–75; morphology and behavior, 75–84; differentiated from E. dysenteriae, 75; from the mouth, description of, 76; living cultural forms, 77; cultural forms from stained slides, 78; behavior in culture, 84; relationship of hydrogen-ion concentration to amoeba cultures, 86–96; determination of pH range for growth of, 89; growth curve, 93; effect of omission of salts from Locke's solution, 96–101; addition of glucose to sugar-free medium, 101; effect of lipoids on cultures, 102–107; cod liver oil added to cultures, 104; addition of cholesterol, 107; addition of protein and amino acids to different media, 107–111; Locke-Lewis medium, 107; experiments with peptone, cystin, 108; tyrosin, 110; addition of blood to cultures, 111–116; effect of low temperatures on growth, 116; summary, 118; methods of cultivation, 174; drug experiments: stovarsol, arsphenamine, neoarsphenamine, and sulfarsphenamine, 176; acetylarsan, emetin-hydrochloride, yatren purissimum, 177; dye experiments, acriflavine, gentian violet, 178; experiments in mixed bacterial cultures, 183–184; growth, in filtered saliva, 184; in media having a solid base, 186; behavior toward peritoneal cells of guinea pig, 189; effect of gastric and duodenal fluids and of bile upon E. gingivalis *in vitro*, 193; ingestion of leucocytes in, 251; material and technique, morphology, ectoplasm and endoplasm in active amoebae, pseudopodia, 252; rounded up amoebae, endoplasm, ectoplasm, nucleus, in unstained amoebae, 253; ectoplasm, endoplasm, pellicle, in stained amoebae, 253; food bodies: leucocytic, of leucocytic nuclei, digestion, stainability, and size, 253; food of E. gingivalis, 254, 255; pathogenicity, 255–258; ingestion of salivary corpuscles, 256–258; digestion of leucocytic nuclei, 256; siderophile extrusions, 259–261; mitosis, literature on, 261—262; resting stage, 263–265; figured, 264; prophase, 266–269; metaphase, 269–270; anaphase, telophase, 270–271.

Endamoeba dysenteriae: In Mixed Cultures with Bacteria, The Behavior of, 127–154; **In Vitro, Studies on the Effects of Certain Drugs upon,** 155–166.

Endamoeba dysenteriae, efforts at cultivation of, 66; behavior in mixed cultures with bacteria, 127; culture medium, 128; culture from chronic cases, 129; egg-Locke-albumen medium, 129; culture from cysts, 132; continuous propagation in artificial media, 133; effect of omission of different ingredients of Locke's solution, 135; growth on different culture media, 137; effect of culturing on coagulated white of egg with Sudan III in cod liver oil, 138; growth in media containing oils, 139; effect of the hydrogen-ion content of the medium on growth, 140; comparative growth on media containing serum, haemoglobin, or whole blood, 142; effect of bile on E. dysenteriae in cultures of coagulated egg-Locke-albumen, 144; growth in media containing varying amounts of cholesterol, 145; effect of methylene blue on E. dysenteriae and the bacteria with which it is associated, 147; effect of acriflavine added to cultures on E. L. B., 147; effects of certain drugs upon, *in vitro*, 155; emetin hydrochloride, 157; acetylarsan, 158; stovarsol, 159; yatren purissimum and yatren casein, 160; neo-arsphenamine, 161; arsphenamine, 162; sulfarsphenamine, 163; mercurochrome, 164.

Entz, cited on unipolar splitting of chromosomes in Ceratium, 37, 45; conjugation, 48.

Essenberg, Christine E., 399–525.

Euglena gracilis Klebs, Mitochondria in, 217–222.

Euglena gracilis, morphology of the motile and encysted phases, 218; mitochondria in the motile and encysted phases, 219, 220; in Noctiluca scintillans, 225–230.

Experiments with Endamoeba gingivalis (Gros) in Bacterial Cultures; in Filtered Saliva; on a Solid Base; with Peritoneal Cells; and with Digestive Secretions, 183–202.

Felis capensis phillipsi, host of Dipylidium sp. of Kofend, 335.

Flagellates, significance of granules of neuromotor system, 238.

Fraser, C. McLean, 167–172.

Fritillaria, 418.
- **amygdala,** 426.
- **angularis,** 437.
- **artus,** 447.
- borealis, 434.
- **brevicollis,** 477.
- **campila,** 445.
- **claudaria,** 478.
- **clava,** 459.
- **delicata,** 475.
- **diafana,** 440.

dispara, 472.
exilis, 430.
formica, 480.
gigas, 464.
haplostoma, 442.
inverta, 423.
juncea, 452.
limpida, 450.
lohmanni, 424.
lucibila, 448.
macrotrachela, 438.
nitida, 458.
pellucida, 460.
plana, 453.
pulchrituda, 437.
ritteri, 455.
sargassi, 468.
tacita, 465.
tenebra, 419.
tereta, 473.
trigonia, 432.
truncata, 471.
velocita, 467.
venusta, 428.
Genetta dongolana, host of Dipylidium dongolense, 319.
tigrina, host of D. gervaisi, 319, 332.
Golgi apparatus, 5; function of, 6.
Gyps kolbi, host of Dipylidium arvicola, 319, 333.
Halicornaria simosa, 171.
Hall, R. P., 29–64.
Halysis genettae. *See* Dipylidium genettae, 318.
Hawk, P. B., cited, 104.
Herpetomonas ctenocephali, resemblance to Leishmania, 365; comparison of morphology, 366, 367; cultural forms of, 367, 368; immunological reactions with antiserums of, 368; agglutination reactions, 371; skin tests with, 373; skin reactions, 376; attempted infection of mice with, 377.
Herpetomonas jaculum, 377.
Howitt, Beatrice F., 65–126, 173–182, 183–202.
Hydroids, New and Unreported, Mainly Californian, 167–172.
Hygrotrechus, 377.
Hymenolepididae, family, 318.
Jollos, cited on centriole in karyosome of Ceratium tripos, 36; on nuclear division, 45.
Kalotermes **arizonensis**, soldier, described, type locality, 393; found with K. minor Hagen(?), 394; holotype, 394; listed, 396.
banksi, 392.
(Glyptotermes) perparverus, 286.
hubbardi, listed, 396.
lighti, soldier, described, 395; type locality, holotype, listed, 396.
(Lobitermes) nigriceps, 296.
minor forma **varius**, 394.
occidentalis, 389.
(Rugitermes) magninotus, 297.
simplicicornis, 391.
Katabolic activity in protoplasmic movements, 239.
Koch, Dorothy A., 365–388.
Kofoid, C. A., 127–154, 155–166, 203–216, 285–300, 301–316.
Kowalevskia, 411.
tenuis, 412.
Lafoea adnata, 170.
Lauterborn, R., account of: division in Ceratium hirundinella, 30, 44; unipolar splitting of chromosomes, 37.
Leishmania and Certain Herpetomonads, The Biological Relationships of, 365–388.
Leishmania, resemblance to herpetomonads, 365; comparison of morphology, 366, 367; cultural forms of, 367, 368; immunological reactions with antiserums of, 368; agglutination reactions, 370; skin tests with cultures of, 373; skin reactions, 375; attempted infection of mice with, 377.
Leishmania brasiliensis, 368.
Vianna, 19; morphology of motile phase, number of mitochondria present, 21; number of mitochondria in cyst of, parabasal body and mitochondria, 23.
donovani (Peking strain), 368.
donovani (Tunis strain), 368.
infantum, 368.
tropica, 368, 377.
Leptocoris trivitattus, 377.
Leucocytes, ingestion by amoebae, 254–258; ingestion of amoebae, 258–259; stainability in iron haematoxylin, 257.
Leucotermes, aureus, 389.
Light, S. F., intestinal smears made by, 302.
Literature cited: Biological relationships of Leishmania, 380; Cestodes, 342; Earliest blood vessels of mammalian embryo, 364; Endamoeba dysenteriae, 149, 166; Endamoeba gingivalis, 12, 121, 182, 199, 273; Mitochondria, 12, 25, 243; Mitosis in Ceratium hirundinella, 52; Modification of Spalteholz clearing fluid, 360; Oxymonas, 299; Oxyphysis oxytoxoides, 215; Proboscidiella multinucleata, 313; Copelata, 519.
Mastigophora, neuromotor system, 44.

Melagocercus, 507.
  diegensis, 508.
Megalotis cerdo, host of Dipylidium echinorhyncoides, 318, 331.
Mice, protozoan infection of, attempted experimental, 377–379.
Millzner, Theresa M., 317–356.
Mitochondria, technique for study of, 3, 217, 226, 232; distribution and function, 3, 4, 7, 10; rôle in amoeboid movement, Brownian movement, 8; number in Leishmania brasiliensis, 21; number in cyst of L. brasiliensis, 23; parabasal body and mitochondria in L brasiliensis, 23; in flagellates, 19, 217; relation to the metabolic activities of Noctiluca scintillans, 227; present in Notiluca, 227; in ciliates, 232; in Paramecium caudatum, 233; in the vegetative organisms, 234; during binary fission, 235; during conjugation, 236.
Mitochondria and Golgi Bodies in Endamoeba gingivalis (Gros) Brumpt, 1–14.
Mitochondria in Euglena gracilis Klebs, 217–224.
Mitochondria in Ciliates with Especial Reference to Paramecium caudatum Ehr., 231–250.
Mitochondria in Leishmania brasiliensis Vianna, 1911, 19–28.
Mitochondria in Noctiluca scintillans (Macartney, 1810), 225–230.
Monocercomonas, 293.
Neotermes connexus, 294.
Noctiluca, mitosis in, 39.
Obelia bicuspidata, 172.
Oikopleura, 483.
  albicans, 498.
  californica, 500.
  cophocerca, 489.
  dioica, 484.
  fusiformis, 494.
  labradoriensis, 502.
  longicauda, 487.
  rufescens, 491.
  vanhöffeni, 496.
Oil of wintergreen, 358, 361.
Oxymonas, a Flagellate with an Extensile and Retractile Proboscis from Kalotermes from British Guiana, 285.
Oxymonas, 295.
  projector, occurrence, 286; general morphology, proportions, 286; cytosome, nucleus, karyosome, bilobed body, neuromotor system, blepharoplast, anterior flagella, 289; rhizoplast, axostyle, axostylar rhizoplasts, 290; proboscis, fibrillar sleeve, 291, 293, 304; retractor fibrils, 291; evolutionary development and relationships, 293; generic description, 295.
  granulosa, 294.
  pediculosa, 296.
  gracilis, 297.
Oxyphysis, general diagnosis, 205.
  oxytoxoides, body, 205; epitheca, girdle, cingular lists, hypotheca, 206; sulcal lists, fission rib, fission suture, sulcus, thecal wall, 207; dimensions, cell contents, megacytic stage, convergence of genus towards Oxytoxum, 208; not a hybrid, 211; specific organ-forming substances, 213.
Oxyphysis oxytoxoides gen. nov., sp. nov., 203–216.
Oxyrrhis marina, nuclear division, 46.
Paramecium caudatum Ehr., mitochondria in, 231–246.
Pennaria tiarella, 172.
Planocryptotermes nocens, 302; a termite from British Guiana, 312.
Proboscidiella multinucleata gen. nov., sp. nov., from Planocryptotermes nocens from the Philippine Islands, a Multinucleate Flagellate with a Remarkable Organ of Attachment, 301–316.
Proboscidiella **multinucleata**, body, cytoplasm, funnel-shaped structures, 302; nucleus, karyosome, number of nuclei, 304; neuromotor system compared with Oxymonas and Giardia, centrosome, rhizoplast, blepharoplast, primary and secondary flagella, proboscis fibers, retractor fibers, 306; number of neuromotor units, proboscis, 307; binary fission, 308; encystment, 309; organization and integration, binomics, 310; relationships and evolutionary origin, 311; cells of Monocercomonas type, 311; a multinucleate xylophagous flagellate, 312.
Protoplasmic movements, katabolic activity in, 239.
Reagan, F. P., 357–364.
Reticulitermes claripennis, 397.
  hoferi, 392, 397.
  humilis, 397.
  tibialis, 397.
  tuniceps, 397.
Rhinotermitidae, 397.
Robinson, A., cited, 362.
Sabin, clearing technique, 357.
Schneider, H., cited on Ceratium tripos, 40, 45.

Scripps Institution of Biological Research, specimen of new Dinoflagellata, 205.
Snyder, T. E., 389–397.
Spalteholz, clearing fluid, modification of, 357.
Stegosoma, 504.
  magnum, 505.
Stephanonympha, 301.
Stomatological Research Group, California, method of diagnosis for Endamoeba gingivalis, 73.
Sudan III stain applied to amoebae, 103–105.
Swezy, Olive, 285–300, 301–316.
Taenia canina. *See* Dipylidium caninum.
  cucumerina. *See* D. caninum.
  echinorhyncoides. *See* D. echinorhyncoides.
  elliptica. *See* D. caninum.
Termites from Arizona with Descriptions of Two New Species, Notes on, 389–397.
Termites, species in the United States, 389; habitations, 389, 390; protozoa of, 390; list of, collected in Arizona, 396; list of known species from Arizona, 397.
Termitidae, 397.
Termopsis laticeps, 389, 397.
Tenuirostritermes tenuirostris, 397.
Trichomonas, 290.
  augusta, 309.
  hominis, 130.
Turritopsis nutricula, 172.
Trypanosoma, skin tests with cultures of, 373.
Vein, primitive anterior cardinal, 362.
Viverra civetta, host of Dipylidium triseriale, 319; host of D. monoöphoron, 332.
  genetta, host of D. genettae and D. triseriale, 332.
Wagener, Edna H., 127–166, 365–388.
Zederbauer, E., "'*Kopulation*'' process in Ceratium hirundinella, 48.

ERRATA

Page 38, line 4. *For* Borget *read* Borgert.

Page 205, line 15 from bottom. *For* General *read* Generic.

Page 293, last line and line 13 from bottom. *For* Stephanonympha *read* Calonympha.

Page 293, line 12 from bottom. *For* to *read* in.

Page 298. Omit lines 10 and 11 from bottom.

Page 298, line 6 from bottom. *For* Stephanonympha *read* Calonympha.

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Page 312, line 8 from bottom. Omit first four words.

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Page 365, line 2 from bottom. *For* Yamasake *read* Yamasaki.

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